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Suppressor of cytokine signaling proteins as regulators of innate immune signaling

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1. ABSTRACT

The innate immune system builds up the body's first line of defense against invading pathogenic microorganisms. For effective defense of pathogenic invaders, a structured inflammatory reaction has to be initiated that is strongly dependent on cell-to-cell communication. Inflammation in turn is a potentially autodestructive reaction that is tightly controlled to balance antimicrobial activity and host damage. Suppressor of cytokine signaling (SOCS) proteins have been identified as crucial negative regulators of various hematopoietic cytokines employing Janus kinas (JAK) and signal transducer and activator of transcription (STAT) signaling. Further results now imply that also signaling by pattern recognition receptors (PRR) of the innate immune system that use a distinct signaling cascade induce and get regulated by SOCS proteins. Thus, SOCS proteins not only modulate cell communication through JAK/STAT dependent cytokines but also regulate signaling by pattern recognition receptors including the Toll-like receptors (TLRs). A model is presented that integrates the current, partly conflicting, data on the role of SOCS proteins in innate immunity's NFkappaB signaling.

2. INTRODUCTION

Based upon the work of Charles Janeway in the mid nineties on pattern recognition, innate immunity since then has experienced a remarkable revival (1,2). Nowadays, we have a fairly detailed understanding of the recognition processes that mediate self/foreign discrimination in innate immune cells, including granulocytes, macrophages and dendritic cells. Central to this is the identification of a (still growing) number of pattern recognition receptors (PRR) (3,4). Four groups of proteins have been recognized that are important in activating a proinflammatory response within innate immune cells: Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD like receptors (NLRs) and RIG-l like receptors (RLRs) which either recognize microbial patterns extracellularly or in cytosolic compartments (3,5). More recently, AIM2 and related receptors ("ALR") have been suggested as an additional group that recognizes microbial nucleic acids (6,7). All receptors make use of central adaptor proteins on which signaling cascades converge (e.g. MyD88/TRIF for TLRs, CARD9 for CLRs, IPS1 for RLRs) finally triggering a few major transcription factors (mainly NFkappaB and IRF family members) that eventually induce soluble immune

mediators like type I IFN, proinflammatory cytokines like IL-6, IL-8, IL-12, TNF or processing of bioactive IL-1. Furthermore, innate immune cells increase their antimicrobial activity including phagocytosis, microbial killing and antigen presentation. In turn an inflammatory reaction is induced. However, effector principles not only target microbial structures but might also harm host cells. In addition, a deregulated magnitude of this response at the end can result in severe host damage as an unwanted side effect (with systemic inflammatory response syndrome as the maximum response). Careful control of the activation status of those cells is thus mandatory and includes activating as well as inhibitory regulatory mechanisms (8).

Suppressor of cytokine signaling (SOCS) proteins have been identified in 1997 as inducible feedback inhibitors of various hematopoietic cytokines triggering the JAK/STAT signaling pathway (9-12). Within resting innate immune cells SOCS genes are transcribed at very low rates, yet their transcription is rapidly induced upon cytokine receptor triggering (13). SOCS proteins can terminate ongoing signaling by the activating receptor (feedback mode) but in some cases the activating pathway is not affected (e. g. IL-10/SOCS3). Moreover, SOCS proteins can regulate the cell's sensitivity towards further cytokines (crosstalk mode). This is because SOCS proteins do not have specificity for only one cytokine receptor. Such a mode of adjusting innate immunity's sensitivity threshold affects activation of innate immunity e.g. by suppressing IFNgamma or IL-6 signal transduction. Work of different groups has clearly shown that SOCS proteins can also be induced in a manner independent of JAK/STAT signaling and pattern recognition receptors increase SOCS transcription rates (14-18). Moreover, sophisticated experiments have now led to the notion that SOCS proteins themselves also contribute to regulation of pattern recognition receptor (mainly TLR) signaling (19-23) or related IL-1 signaling (24,25). Recent work has especially centered on the role of SOCS proteins in the regulation of the major transcription factor NFkappaB within innate immune cells (26-29).

3. STRUCTURE OF THE SOCS FAMILY

The SOCS family consists of eight family members (Cytokine inducible SH2 containing protein CIS, SOCS1-7) which in principal share a similar build-up (13,30). All SOCS proteins possess a central SH2 region that allows for binding of phosphotyrosine residues within type I and II cytokine receptor domains or within Janus kinases. This protein interaction site therefore enables access of SOCS proteins to activated cytokine receptors or JAKs. In turn, this interaction can block further signaling through competition for STAT recruitment. This mode of action has been reported for CIS and SOCS2 that both activate STAT5, e.g. via IL-2, IL-3, erythropoietin, or growth hormone (GH) (12,31-33). Indeed, SOCS2 knockout mice have a defect in limiting GH/STAT5 signaling (34). SOCS1 and SOCS3, which are strongly regulated by PRR activation, possess an additional protein interaction domain. The kinase inhibitory region (KIR) is adjacent to the extended SH2 region and has been proposed to act as a pseudosubstrate for JAKs thus inhibiting JAK tyrosine kinase activity (35). A protein interaction model predicts direct binding of SOCS1 to the activation loop of JAK2 through the SH2 domain (36). In contrast SOCS3 gets access to JAKs via binding to the cytokine receptor as exemplified by binding to the IL-6 receptor/gp130 (37,38), erythropoietin receptor (32), G-CSF receptor (39), leptin receptor (40,41) or growth hormone receptor (42). SOCS mimetics that also inhibit JAK activity have been identified and could be of use to mimic the effects of SOCS proteins (43-45). All SOCS proteins additionally bear a carboxy-terminal SOCS box domain that acts as an E3 ubiquitin ligase (46-48). Thus, the SOCS box recruits elongin B and C, cullins and Rbx-1 and mediates poly-ubiquitination and proteasomal degradation of bound proteins. Whereas KIR and SH2 domains contribute to specific inhibitory activities, the SOCS box assigns a further, more general inhibitory activity to SOCS proteins. Indeed, a contribution of the SOCS box to the overall biology of SOCS proteins has been confirmed: Upon blockade of the proteasomal degradation machinery *in vitro* SOCS proteins are less inhibitory. Moreover, the disease phenotype of mice lacking the SOCS box of SOCS1 is ameliorated as compared to full SOCS1 knockouts (49). On the other hand, SOCS3 itself gets stabilized by interacting with elongin C (50) and phosphorylation of a SOCS box tyrosine residue decreases this interaction and in turn increases protein turnover. Thus, SOCS1 and SOCS3 are regulated by SOCS box interaction themselves (51). Indeed, stability of SOCS proteins seems low with half-life times of few hours at maximum (45,52).

Recent work has identified an additional feature specifically found in SOCS1. SOCS1 but none of the others SOCS proteins showed a predominant nuclear localization when YFP- or CFP tagged fusion proteins were examined (53). In the same line overexpressed myc-tagged SOCS1 accumulated within the nucleus in a study analyzing the interaction with human papilloma virus E7 protein (54). Additionally, it was observed that SOCS1 within the nuclear fraction of NIH-SR cells contributes to NFkappaB ubiquitination (28) and SOCS1 was also found in the nucleus of transfected COS, 293T and Jurkat cells (55). Thus, evidence accumulated that SOCS1 might localize to the cell nucleus implying additional functions in this compartment. In fact, SOCS1 was also reported to be expressed in the nucleus in human keratinocytes within an inflammatory surrounding environment and it was speculated about different functional properties of nuclear vs. cytoplasmic SOCS1 (e.g. modulating the stability of transcription factors) (56). However, the expression pattern initially remained disputed as one group stated that glucocorticoid receptor binding drives nuclear translocation of SOCS1 (57) whereas others attributed the unexpected localization to protein overexpression (58) or focused on colocalization of SOCS1 with the microtubule organizing complex and 20S proteasome (55). In a series of experiments our group showed that SOCS1 bears a specific nuclear localization signal (NLS) (59,60) that followed the consensus rules of classical bipartite NLS (61). Besides a membranous expression pattern, fluorescent fusion constructs of SOCS1 as well as endogenous SOCS1 itself

was found in the nuclear compartment in a variety of cells. Mutating the basic residues within the NLS sequence abolished nuclear localization and ectopic expression of the SOCS1-NLS sequence in otherwise cytoplasmic CIS induced nuclear localization. SOCS1 was shown to be highly mobile in the nucleus with half-time of recovery in FRAP experiments being about 10 s (59). These results thus identified and verified a functional NLS in SOCS1 and point towards a so far unrecognized function for nuclear SOCS1. Single reports also state that SOCS7 translocates to the nucleus due to a monopartite NLS (62) and SOCS6 also could be observed in the nucleus (63). Very recently, SOCS3 was also shown to be expressed in the nucleus as well as the cytoplasm of macrophages, epithelial cells and endothelial cells in various tissues with more intense staining during inflammation (64). However, confirmation of those results as well as evaluation of their biological significance is still lacking. Taken together, those results in any case indicate that the functional properties of SOCS are still not fully uncovered.

4. SOCS AND MACROPHAGE/DENDRITIC CELL BIOLOGY

SOCS1, SOCS3 and CIS proteins can be induced in cells of the innate immune system through different routes, either via JAK/STAT dependent cytokines or via pattern recognition receptors (outlined below). In turn SOCS can inhibit the inducing pathway in a classical feedback manner or adjust the sensitivity towards further SOCS sensitive signals (crosstalk inhibition). The importance of SOCS proteins in innate immune cells has been addressed by genetic means.

SOCS1 knockout mice succumb to perinatal death within the first weeks which is due to liver necrosis, lymphopenia, and unregulated T-cell activation (65-68). Moreover, macrophage infiltrations can be observed in various organs pointing towards a crucial role of SOCS1 for homeostasis of macrophages. As the concomitant lack of IFNgamma or the IFNgamma receptor (65,66,69) ameliorates the disease it was concluded that the phenotype is mainly due to lack of IFNgamma signal termination. T-cell derived IFNgamma is thus not limited anymore with respect to its macrophage stimulating activities. Indeed, lack of T cells also rescues the immediate early disease phenotype. However, IL-4 and IL-12 signaling was also disturbed as evidenced by backcrossing of SOCS1 to STAT4 or STAT6 knockout mice. Moreover, SOCS1/IFNgamma double knockout mice showed a delayed disease phenotype with reduced lifespan, polycystic kidneys and chronic granulomas in various organs (70) arguing for a more complex disease pathology with acute and chronic inflammation in SOCS1 knockout mice. This might include disturbed signaling by IL-2 (71,72), IL-6 (73), IL-12 (74), IL-15 (75) or TLRs and TNFR (19-22,76). Macrophages and DCs among other cells also express SOCS proteins and SOCS proteins within those innate immune cells exert inhibitory activities. Thus it might very well be that the latter cells are also involved in the direct development of the disease. Indeed, SOCS1/IFNgamma double knockout mice show increased sensitivity towards stimulation by LPS (19,20) with elevated secretion of innate cytokines (TNF, IL-12).

Importance of SOCS1 for DC biology was first studied in SOCS1 deficient mice restored with SOCS1-expressing T- and B cells. In those mice DCs accumulated in spleen and thymus producing B-lymphoproliferative cytokines that expanded the B-cell pool and finally induced autoantibodies (77). It was concluded that SOCS1 is necessary in DCs to suppress autoimmunity. Breakdown of tolerance was also observed when SOCS1 siRNA treated DCs were used which produced more IL-12 (78). Besides a role of SOCS1 for the function of DCs it was also shown that DC development is regulated by SOCS1. Thus, SOCS1 was shown to inhibit GM-CSF mediated differentiation of monocytes to DCs (79). It was also suggested that SOCS1 plays a restrictive role in the switch from STAT6 to STAT1 in the process of DC maturation (80). A role of SOCS1 in the development of DCs was further substantiated by the observation that miR-155 regulates DC apoptosis and IL-12 secretion and SOCS1 is a validated miR-155 target (81). Moreover, lower SOCS1 expression also correlated with the increased secretion of IL-12 by CD11c/CD8a$^+$ DC subset and SOCS1 deficient mice contain higher numbers of CD8a$^+$ DCs which express more MHC class II and costimulatory molecules (77,82). Findings on IL-12 were further substantiated by the observation that IL-12 secretion was elevated upon siRNA mediated knockdown of SOCS1 (83). Silencing of SOCS1 within DCs led to increased intestinal allograft survival (84). Reduced IL-12 was also observed in monocytes/macrophages triggered by HCV core protein to increase endogenous SOCS1 levels (85).

SOCS3 has been analyzed in detail with respect to its function in macrophages. It mainly affects sensitivity of macrophages towards gp130 related cytokines. Upon IL-6 stimulation SOCS3 conditional knockout macrophages showed increased induction of STAT1 instead of STAT3 dependent genes. In turn a STAT1 shifted gene expression profile was observed that resembled the activities of IFNgamma (86,87). It was concluded that SOCS3 is important to shape the nature of a specific IL-6 response in macrophages. Furthermore, SOCS3 deletion resulted in a prolonged STAT3 activation which was not limited appropriately anymore and consequently mimicked some inhibitory actions otherwise observed for IL-10 (88). The latter cytokine typically induces STAT3 but is insensitive to SOCS feedback inhibition (89). A similar phenotype was observed for macrophages lacking the SOCS3 binding site (Y759F) in gp130 (88). However, this finding which was discussed to confirm the previous experiments has to be interpreted with caution because this mutation also abolishes MAP kinase activation by IL-6 (90). Taken together, SOCS3 is doubtlessly important to regulate the opposing functions of IL-6 and IL-10 that mediate pro- or anti-inflammatory activity through the same STAT3 dependent signaling pathway but differ with respect to the signaling kinetics. Consistently, SOCS3 knockout macrophages were more resistant in the LPS shock model, which is opposite to the results in STAT3 conditional knockout mice (91) and supports the interpretation of SOCS3 as a suppressor of macrophage and DC activity

(23). SOCS3 additionally plays an important role to limit G-CSF and GM-CSF (92) thereby regulating myeloid cell differentiation. SOCS3 regulates $CD11c^{+}$ DC mediated osteoclastogenesis, a condition that might be of importance in periodontal disease (93). Within macrophages it has been shown that SOCS3 is necessary to drive differentiation into the classical M1 phenotype, including suppression of IL-4 signaling, whereas SOCS3 knockdown resulted in alternative M2 differentiation (94). With respect to DC differentiation SOCS2 upregulation has also been reported and SOCS knockdown interfered with DC maturation including reduced expression of co-stimulatory molecules and reduced LPS signal transduction (95).

5. SOCS INDUCTION BY TLR STIMULATION

Within macrophages and dendritic cells mainly SOCS1, SOCS3 and CIS are regulated upon microbial encounter. Transcription under resting conditions is low but increases rapidly upon stimulation of various PRRs. Rapid induction is ascribed to the gene structure of SOCS genes with only few introns. Besides transcriptional regulation SOCS1 is controlled through translational repression due to an additional start codon in the 5' region (96). Similar observations have also been reported for an N-terminal truncated isoform of SOCS3 (97). As pointed out above protein stability of SOCS1 and 3 themselves is also regulated. During sepsis, SOCS3 is mainly up-regulated in macrophages and neutrophils thus hinting towards an important function in innate immunity (98).

Whereas SOCS molecules have been shown to be regulated by various cytokines acting through the JAK/STAT pathway in innate immune cells, including IFNgamma, IL-6 and IL-10 it was also reported that TLR triggering up-regulates transcriptional levels of SOCS3 and SOCS1, the latter with slightly slower kinetics. This was observed initially for LPS/TLR4 (14) and then confirmed for many more of the TLRs (15,99). Beside TLR stimulation, activation through TNF also results in SOCS3 induction (16) and TNF can further enhance LPS-induced SOCS3 (100). Another PRR, DC-SIGNR1 which is important in mycobacteria recognition, has also been shown to induce SOCS1 thereby limiting IL-12 production (83).

SOCS3 induction in the setting of TLR stimulation is dependent on the central TLR adaptor protein MyD88 whereas SOCS1 showed additional dependence on TRIF for TLR4 but not TLR9 stimulation (22). The initial suggestion that SOCS induction upon TLR triggering was indirect due to the intermediate secretion of IFNbeta (101) has been rejected, because TLR stimulation induces SOCS1 and SOCS3 independent of intermediate protein synthesis, small secreted factors and without alteration in $IFNAR^{-/-}$ macrophages (22). For TLR mediated induction of SOCS proteins MAP kinase activation (p38) is necessary (15) and this is supported by the observation that MAPK activation itself is sufficient to drive SOCS3 transcription (102). Although STAT binding elements in general are important for SOCS induction (reflecting negative feedback regulation and eponymous for STAT-induced STAT inhibitor, SSI, syn. of SOCS) the activation of SOCS1 by LPS has been shown to be due to early-growth response factor-1 (103). SOCS3 induction by LPS was shown to be dependent on AP1 as well as GAS elements within the promoter and furthermore, at later time points, secondary IL-10 contributed to prolonged induction (104). Moreover, it was shown that TNF augments SOCS3 stability through tyrosine phosphorylation (100) as well as mRNA stabilization (105) thus once again emphasizing the importance of posttranscriptional and posttranslational regulation.

6. MODES OF ACTION OF SOCS PROTEINS IN TLR SIGNALING

SOCS proteins can be induced through pattern recognition receptors in innate immune cells. Therefore it was not surprising to see that SOCS dependent JAK/STAT cytokine signaling was modulated in a SOCS dependent manner upon TLR stimulation. This is exemplified by the observation that within macrophages SOCS1 and SOCS3 are induced upon TLR triggering and in turn inhibit signaling by subsequently added IFNgamma resulting in decreased STAT1 phosphorylation and gene induction (14,15). Such a mode of crosstalk inhibition in which a TLR signal results in down-regulation of an independent JAK/STAT dependent signal can be misused by pathogens including Leishmania (106,107), mycobacteria (108,109), Toxoplasma (110), HIV (111,112) and Respiratory Syncitial Virus (113-115). Those pathogens trigger pattern recognition receptors including TLRs and induce SOCS proteins which in turn inhibit the detrimental activities of IFNgamma on pathogen survival in macrophages. SOCS1 and SOCS3 play an important role in fine-tuning the balance of avoidance of infection-induced inflammation without impairment of pathogen control as shown for Leishmania and chlamydia (106,116). In a similar manner it was shown that SOCS induction through TLRs interferes with additional cytokine pathways including GM-CSF/STAT5. SOCS proteins inhibited GM-CSF activity thereby interfering with the differentiation of dendritic cells (79,117).

Based upon the observation that SOCS1, SOCS3 and CIS are induced through TLR stimulation the question arose whether SOCS proteins themselves would also be able to interfere with TLR signaling. Indeed, two groups independently observed (19,20) that SOCS1 knockout mice showed elevated cytokine levels and increased toxicity *in vivo* upon LPS stimulation. A contribution of increased SOCS transcript levels towards the condition of LPS hyporesponsiveness/tolerance (118) was also reported in the context of CD14 signaling (119). From these observations it was concluded that SOCS1 is an important restriction factor for TLR signaling and it was suggested that NFkappaB activity is regulated. However, the underlying mechanism remained elusive and only a weak interaction of SOCS1 with IRAK1 via the SH2 domain (19) could be detected. Subsequent work using SOCS overexpression in RAW264.7 macrophages did not confirm a direct inhibition of TLR signaling by SOCS1. TNF secretion as well as NFkappaB reporter gene activity was

not altered. However, IP10, a gene known to be induced in an indirect manner upon TLR stimulation, was reduced in SOCS1 overexpressing cells (22). This observation led to the conclusion that SOCS1 indirectly regulates TLR signaling through inhibition of autocrine or paracrine IFNbeta signaling. Indeed, TLR4 and -9 stimulation induced IFNbeta resulting in secondary STAT1 tyrosine phosphorylation. SOCS1 overexpression did not affect primary IFNbeta induction, yet reduced IFNbeta stimulated STAT1 activity. MAP kinase activities and induction of NFkappaB dependent genes (TNF, IL-6) were not affected. This notion of an indirect action of SOCS1 was confirmed by *in vivo* data showing that hypersensitivity of SOCS1 mice towards LPS stimulation vanished in SOCS1/Tyk2 double knockout mice (21). The observations support the concept that TLR stimulation induces IFNbeta that in turn activates Tyk2 which increases transcription rates of a subset of TLR induced genes (secondary genes) (120,121) thus representing an important amplification cascade in TLR signaling (122). Very recently it was shown that glucocorticoids limit secondary, delayed TLR signaling including STAT1 phosphorylation through induction of SOCS1, a mechanism that fits exactly to the described mode of action of SOCS1 in TLR signaling (123). However, the situation might be even more complex as discussed in more detail below.

Additional experiments have also shown that TLR4 stimulation activated JAK2 and this axis was regulated through SOCS1 in innate immune cells (124). SOCS1 was also shown to limit JNK activation through degradation of ASK (125). Further observations confirm that SOCS proteins, beside their well defined activity in JAK/STAT signaling, affect pattern recognition receptors as well. SOCS1 has been shown to ubiquitinate TIRAP/Mal, an adaptor protein used in TLR2 and TLR4 signal transduction, through its SOCS box. This modification of TIRAP/MAL occurred upon Bruton's tyrosine kinase dependent phosphorylation and resulted in proteasomal degradation and reduced NFkappaB activity (126). In turn, deletion of SOCS1 led to increased TIRAP/Mal phosphorylation, NFkappaB activation and proinflammatory activity in macrophages. Thus, while doubtlessly SOCS1 modulates TLR signaling, still partly contradictory results are reported with respect to direct manipulation of NFkappaB activity.

Much less is known for SOCS2, SOCS3 and CIS which are also induced in innate immune cells with respect to their role in TLR signaling. SOCS3 and CIS do not seem to directly interfere with PRR signaling (22); however, through modulation of a cell's cytokine sensitivity they also contribute to signal transduction regulation in macrophages and DCs (mostly by crosstalk regulation). For SOCS2 an induction by lipoxin was reported and this contributed to down-regulation of proinflammatory activity through TNF receptor-associated-factor (TRAF) 2 and TRAF6 poly-ubiquitination (127,128). TRAF2 and 6 were then degraded by the proteasome and this correlated with the anti-inflammatory effects of lipoxins.

7. SOCS1 AND NFKAPPAB SIGNALING

NFkappaB is the major transcription factor in TLR signaling (129). NFkappaB is a dimer which is composed out of five different family members: p65/RelA, RelB, c-Rel, p52 and p50. In resting cells the prototypical NFkappaB heterodimer p65/p50 is kept in the cytoplasm by interaction with IkappaBalpha. The latter is phosphorylated and degraded upon TLR stimulation through IkappaB kinase; NFkappaB is then liberated and transported into the nucleus where it initiates gene transcription (130). Among a number of genes IkappaBalpha is re-induced, synthesized and shuttles NFkappaB out of the nucleus again and thus terminates signaling. Besides this canonical pathway of inactivation, it became clear in recent years that additional regulatory pathways that contribute to termination of NFkappaB signaling exist (131,132). For example, p65 stability and nuclear availability is regulated through polyubiquitination and proteasomal degradation within the cell nucleus. PDLIM2 was identified as an ubiquitin ligase involved in this process (133) which also translocates p65 into nuclear PML bodies, sites of nuclear protein regulation. In 2003 it was suggested that also SOCS1, through its activity as E3 ubiquitin ligase, contributed to p65 stability and proteasomal degradation (26). Moreover, an interaction between p65 and SOCS1 dependent on the protein COMMD1 was reported later (28) and COMMD1 inhibited NFkappaB activity. In this line, it was also shown that TNF induced serine phosphorylation at S276 of p65 in a manner dependent on Pim-1 resulting in enhanced transcriptional activity and protection of p65 degradation through SOCS1 (134).

Taken together with the recent observation that SOCS1 can be found in the nuclear compartment (59) we speculated that SOCS1 might interact with nuclear p65 and regulate the stability of the latter. Although initial studies (79) did not show an inhibition of NFkappaB activation by TLR induced SOCS proteins, it subsequently became clear that kinetics of NFkappaB activation are a decisive variable controlling expression in a gene specific manner (135). Therefore we analyzed whether nuclear SOCS1 interacts with p65. Our recent results show that SOCS1 interacts with p65 within the nucleus and that this interaction results in ubiquitination and proteasomal degradation of p65 (27). In turn, the reduced availability of p65 resulted in a decreased induction of NFkappaB dependent genes. Of notion however, only a subset of NFkappaB dependent genes was affected. Confirming these results non-nuclear mutants of SOCS1 showed reduced inhibitory effects on a few NFkappaB dependent genes. The exact mode of binding of SOCS1 to p65 remains to be elucidated but co-activating factors of the NFkappaB enhanceosome like UXT might be involved (136). Own data suggest that the SH2 domain is involved in p65 interaction and whether this is dependent on tyrosine phosphorylation or independent, as reported for binding to E7 (54) or vav (137), is presently unclear. Further support for this model comes from a report claiming that ORF73 from murine herpesvirus-4, encoding for a SOCS box like protein, terminates NFkappaB signaling through ubiquitination and proteasomal degradation thereby representing a mode of immune evasion (138). Importantly, SOCS1 overexpression only reduced the induction of a subset of NFkappaB genes which might finally explain the accumulated controversial results: Thus, the inhibitory effects of SOCS1 on

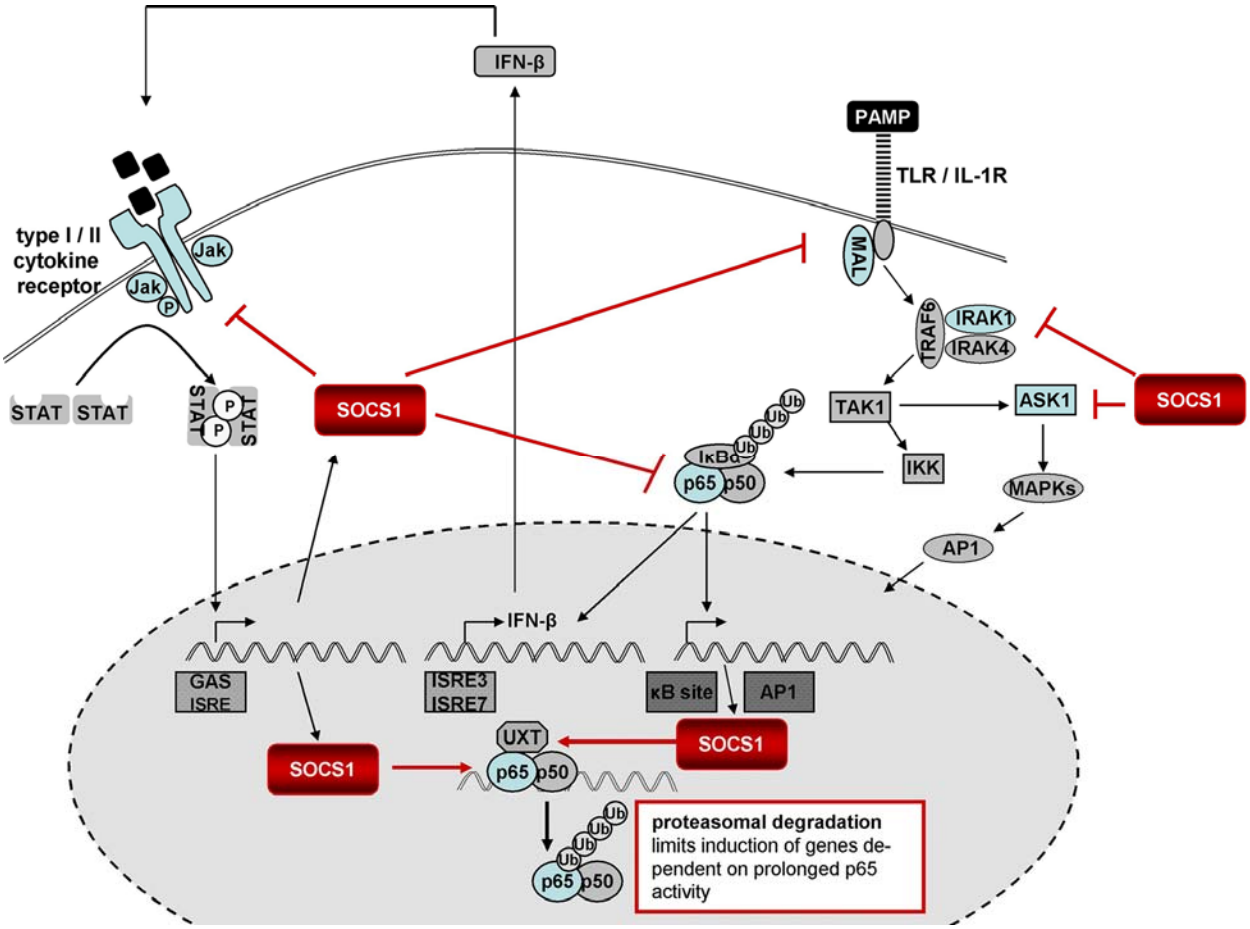

Figure 1. Inhibitory effects of SOCS1 in TLR signaling. SOCS1 expression is directly induced by cytokine or TLR stimulation. SOCS1 inhibits JAK/STAT signaling in a feedback mode or as crosstalk inhibition. Moreover, SOCS1 induces the poly-ubiquitination and proteasomal degradation of the adaptor protein TIRAP/Mal. SOCS1 binds to IRAK1 and thereby abrogates TLR signal transduction. SOCS1 also regulates MAP kinase activation by binding to ASK1 which is an upstream activator. Finally, SOCS1 binds to p65 and facilitates its poly-ubiquitination and subsequent degradation, thus limiting NFkappaB signaling. Degradation of p65 results in decreased nuclear availability thus affecting a subset of p65 dependent genes whose induction is dependent on prolonged NFkappaB activity.

NFkappaB signaling do not affect *induction* of NFkappaB activity but limit the *duration* of its transcriptional activity. In turn, genes that only need a short NFkappaB stimulation are not affected by SOCS1 whereas others, that require more prolonged NFkappaB activation (139), e.g. including extensive chromatin remodeling (secondary genes, (135)), are sensitive towards SOCS1 overexpression. NFkappaB duration can be influenced by nuclear SOCS, but again there are also genes that were equally inhibited by non-nuclear mutants which is compatible with additional reports on further upstream effects. Taken together, SOCS1 seems to affect TLR signaling at different levels of signal transduction which results in gene-specific inhibitory effects (Figure 1).

8. CONCLUSIONS

Within innate immunity SOCS proteins are important inhibitory molecules that limit a cell's sensitivity to signals acting on the cell through the JAK/STAT signal transduction pathway. In turn SOCS proteins balance and adjust the threshold for cytokine activation and contribute to the termination of such signals in innate immune cells. Therefore SOCS proteins regulate cell activation, differentiation and homeostasis as well as microbial defense. Furthermore, SOCS proteins also modulate signaling of innate immunity's pathogen sensing receptors, the pattern recognition receptors. A variety of mechanisms by which SOCS proteins regulate Toll-like receptors has now been deciphered. Among those fine-tuning of NFkappaB is a common theme affecting in turn a subset of NFkappa dependent genes in a cell- and stimulus-dependent manner. Recent results on localization of SOCS proteins suggest that the full activities of SOCS proteins are still not entirely uncovered. Nuclear localization of SOCS1 implies additional regulatory properties which is one of the recent exciting discoveries. In the future it will be of importance to get full insights into those "new" functions of SOCS proteins. Additionally, there is still a lack of understanding of the biology of the less well studied SOCS4-7 proteins.

Besides molecular mechanisms SOCS proteins are increasingly recognized as important regulators in inflammatory diseases. Analyzing the various SOCS knockout mice in inflammatory disease models will further substantiate our understanding of the clinical importance of SOCS proteins. Finally, given the central role of SOCS proteins in JAK/STAT as well as further signaling cascades this protein family bears a potential as drug target and should be evaluated further in this direction.

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Abbreviations: CIS, cytokine inducible SH2 domain containing protein; DC, dendritic cell; IFN, interferon; IL, interleukin; JAK, janus kinas; NFkappaB, nuclear factor kappa B; PRR, pattern recognition receptors; SH2, src homology 2; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TLR, toll-like receptor; TNF, tumor necrosis factor

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