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REGULATION OF SOCS EXPRESSION: A PROMISING TARGET FOR ANTI-HIV RESEARCH

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ABSTRACT

Suppressor of Cytokine Signaling (SOCS) proteins are the negative regulators of cytokine signals as a natural precaution to prevent excessive responses that could cause damage to body tissues. However, their over expression is suggested to inhibit the anti-proliferative and antiviral activities of Interferon (IFN). Several viruses including HIV-1 have been reported to exploit the SOCS proteins for minimizing the activity of Interferon (IFN). Hence, the attempts to regulate the SOCS expression may be a novel roadblock into viral proliferation and pathogenesis. Present review is an effort to summarize the SOCS contribution in viral contagion and future therapeutic prospects against HIV-1. *Running Head*- Aberrant SOCS expression: a target for anti-HIV research

KEY WARDS: HIV-1, Suppressor of Cytokine Signaling (SOCS), Interferon (IFN), JAK/STAT pathway, IL-7

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INTRODUCTION

Current therapies that directly target to virus are often ineffective due to emergence of drug resistance and viral variants. Therefore, targeting to host mechanisms that are essential for the propagation of virus but have a minimal cellular effect is an emerging concept to combat drug resistance. Suppressors of Cytokine Signaling (SOCS) protein family is one amongst the recently identified group of host proteins that can be exploited by virus for their own benefits. Members of this family are induced at infection by a number of different viruses, including HIV-1, Hepatitis C Virus (HCV), Hepatitis B Virus (HBV), Herpes Simplex Virus type 1 (HSV-1) etc. to subsequently contribute in viral replication and pathogenesis (Akhtar and Benveniste, 2011). Normally, SOCS proteins negatively regulate the cytokine signals as a natural precaution to prevent 'out-of-control' responses that could cause collateral damage to body tissues. But over expression of SOCS proteins are suggested to suppress even required immune response added with inhibition of the anti-proliferative and antiviral activities of Interferon (IFN). Interferon is a pleiotropic cytokine produced primarily by T lymphocytes and Natural Killer (NK) cells in response to viral infection (Schroder et al., 2004). It is an important mediator with multiple biological activities such as macrophage activation and, antimicrobial, anti-proliferative and immunomodulatory effects (Samuel, 2001). At cellular level, IFN induce the activation of Janus kinase-1 (Jak1) and Jak2 and consequent phosphorylation of STAT1 which finally leads to initiation of transcription after dimerization (Song and Shuai, 1998; Stoiber et al., 1999; Platanias, 2005). But in AIDS patients, the IFN signaling cascade is inhibited proximally at the level of STAT1 phosphorylation by viral *Tat* protein and therefore opens a window of leeway for opportunistic microbial infection in HIV-infected hosts (Fig-1) (Cheng et al., 2009).

SOCS Protein family

SOCS protein family including SOCS-1 to SOCS-7 and cytokine-inducible SH2 protein (CIS) represents the eight different members (Fig-2). They consist of the inhibitors of activated STATs (PIAS) and Scr-homology 2 (SH2) containing protein tyrosine phosphatases (SHPs) which contribute to the negative regulation of cytokine signaling (Danielle and Douglas, 2000). However, so far only first four proteins; CIS, SOCS-1, SOCS-2 and SOCS-3 are reported to be induced by cytokines. No clear evidences for the cytokine induced expression of SOCS-4, SOCS-5, SOCS-6 and SOCS-7 mRNAs are found. The first four proteins are induced by cytokine stimulation and inhibit to the same cascade initiating their production by a classical negative feedback mechanism (Larsen and Röpke, 2002).

Suppression of cell signaling

A cytokine after binding to their respective receptors cause phosphorylation of the cytoplasmic receptor domain and rise in JAK-STAT phosphorylation (Yoshimura et al., 1995; Endo et al., 1997; Starr et al., 1997). But subsequent SOCS induction seems to be able to stop further signal transduction by inhibiting various steps in this cascade (Fig-1). The over expression of SOCS-1, SOCS-2, SOCS-3 and CIS in cell lines have resulted in reduced phosphorylation of JAKs or STATs (Naka et al., 1997; Starr et al., 1997; Song and Shuai, 1998), diminished STAT dimerisation (Starr et al., 1997), jumbled import of STAT to the nucleus (Song and Shuai, 1998) and finally reduced transcription of target genes (Endo et al., 1997) caused by a wide range of cytokines (Yoshimura et al., 1995; Starr et al., 1997). Suppressor of Cytokine Signalling 1 (SOCS-1) is one of the proteins responsible for negative regulation of JAK-STAT pathway.This involves the complex formation between SOCS-1 and JAK2 through

particular structural domains (KIR, ESS and SH2) present on SOCS-1. JAKs and STATs are the receptor associated tyrosine kinases and cytoplasmic transcription factors which communicate the cytokine effect within the cell (Imada and Leonard, 2000). This complex cascade is crucial for the cell development, hematopoiesis and host defence and requires precise control and regulation. It has been reported that SOCS can modulate the host response to IFN and prevent activation of STAT-1. Moreover, SOCS proteins have also been reported to inhibit the cytokine-induced signaling cascade for ubiquitination of proteins and proteosomal degradation. Hence, SOCS proteins may lead the host to infection, contribute to disease manifestations such as immune dysfunction and cancer, and even modify the efficacy of therapeutic interventions.

Behavior of SOCS proteins in viral infection

It was recognized that viruses could hijack host SOCS proteins to manipulate antiviral IFN signaling for their advantage. Hepatitis C Virus (HCV), a small, positive-sense, single-stranded RNA (ssRNA) virus that infects hepatocytes of the liver to cause liver inflammation (hepatitis), was the first virus reported to independently induce SOCS expression (Bode, 2003). In continuation of that, effect of *Mycobacterium avium* has also been investigated on SOCS members for its inhibiting properties on the activation of IFN-gamma induced STAT-1. *M. avium* is an opportunistic pathogen commonly attacks HIV-1 colonized individuals. It interacts with TLR2 and/or CD14 co-receptors and invades macrophages. This is observed that the *M. avium* in collaboration with macrophage receptors resulting in the elevated gene expressions of the SOCS which further causes diminished IFN responsiveness with suppressed phosphorylation of STAT-1 in *M. avium* infected cells as compared to uninfected cells (Vázquez et al., 2006).

However, numerous viruses are used to “hijack” the SOCS function promoting viral survival, as SOCS proteins inhibit protective antiviral signaling pathways, thus allowing facilitation of viral proteins, intracellular viral trafficking and viral progeny assembly. Hence the SOCS proteins not only allow progression of viral life cycle but also powerfully shape the presentation of viral diseases (Akhtar and Benveniste, 2011). It inhibits the signaling by a wide range of cytokines including interleukin 2 (IL-2) (Sporri et al., 2001), interleukin 4 (IL-4), interleukin 6 (IL-6), leukaemia inhibitory factor (LIF) (Starr et al., 1997), interferon γ (IFN-γ) (Sakamoto et al., 1998), growth hormone (GH) (Ram and Waxman, 1999) and prolactin (PRL) (Tomic et al., 1999).

Moreover, influenza A virus has also been testified for its suppressive/inhibitory effects on interferon (IFN) beta gene by a mechanism comprising generation of the suppressor of cytokine signaling-3 (SOCS-3) protein. Because the influenza A virus infected cells were reported to have strongly condensed phosphorylation of the signal transducer and activator of transcription protein 1 (STAT1) (Pothlichet et al., 2008). This impaired STAT1 activation is believed to appear due to accumulation of viral 5' triphosphate RNA in the cell.

SOCS proteins in HIV-1 infection

The interplay between viruses and host cells determine the outcomes of viral pathogenesis, ranging from elimination of viruses to latent or lethal infections. HIV-1 is not an exception. In fact, individuals with defective cell surface receptor (CCR5) express resistance to HIV-1 infection (Liu et al., 1996; Samson et al., 1996) while soluble mediators including cytokines and viral products such as *Tat,* produced by infected cells augments the HIV-1 infection either by direct effects or through deregulation of cytokine expression viz. Interferon (IFN) and Tumor Necrosis Factor (TNF) (Sui et al., 2009; Wheeler et al., 2007). Several similar interactions have been

reported to encompass nearly every step of HIV-1 life cycle starting from viral entry (Moore, 1997) to viral budding and release (Garrus et al., 2001).

SOCS proteins are induced by viruses including HIV-1 to promote the successful completion of their own life cycle. Its aberrant expression can powerfully shape the overall presentation of viral disease by contributing to the peripheral manifestations of viral infection. For example, the direct result of HIV-1 replication is $CD4^+$ T cell death (Dwivedi et al., 2011) but the most often manifestation of HIV-1 disease may be opportunistic infection by the pathogens such as mycobacteria, protozoa, and fungi. However, these pathogens are cleared in the immunocompetent host primarily by T cell-mediated IFN-γ signaling. But decreased T cell numbers in the HIV-1 infected host certainly hinder proper clearance of them. Even exogenous supply of IFN-γ has not shown any significant effect (Biggs et al., 1995), suggesting that nonproductive signaling is also at fault. It seems likely that HIV-1 induced SOCS1 and SOCS3 proteins provide a safe haven for *M. avium* and other opportunistic pathogens by inhibiting the IFN-γ signaling.

The cytokine expression in HIV positive individual is characterized as the decreased production of pro-inflammatory cytokines i.e. IL-12 and IFN-γ and increased expression of the anti-inflammatory cytokine IL-10. The IFN-γ and IL-12 signaling network is fundamental to the Th1 differentiation program and in controlling immunity against HIV-1 infection (Murphy et al., 2000). Therefore, the absence of IFN-γ and IL-12 and better presence of IL-10 sponsor the impaired innate and T helper type 1 (Th1) responses in AIDS patients (Ma and Montaner, 2000). Moreover, in relation to SOCS support to HIV-1, Wahl and colleagues observed that tonsil mucosal associated lymphoid tissues are much more susceptible to HIV-1 infection than peripheral blood mononuclear cells (PBMCs), due to the increased levels of SOCS1 and SOCS3 present in these tissues (Moutsopoulos et al., 2006). Increased SOCS expression in tonsil tissues, both constitutively and in response to HIV-1 infection, correlated to decrease STAT1 activation in response to IFN-α and a decreased T_H1 response in the presence of IFN-γ. These studies suggest that SOCS expression may contribute to determining susceptibility to HIV-1 infection by inhibiting both the innate and adaptive immune responses to infection.

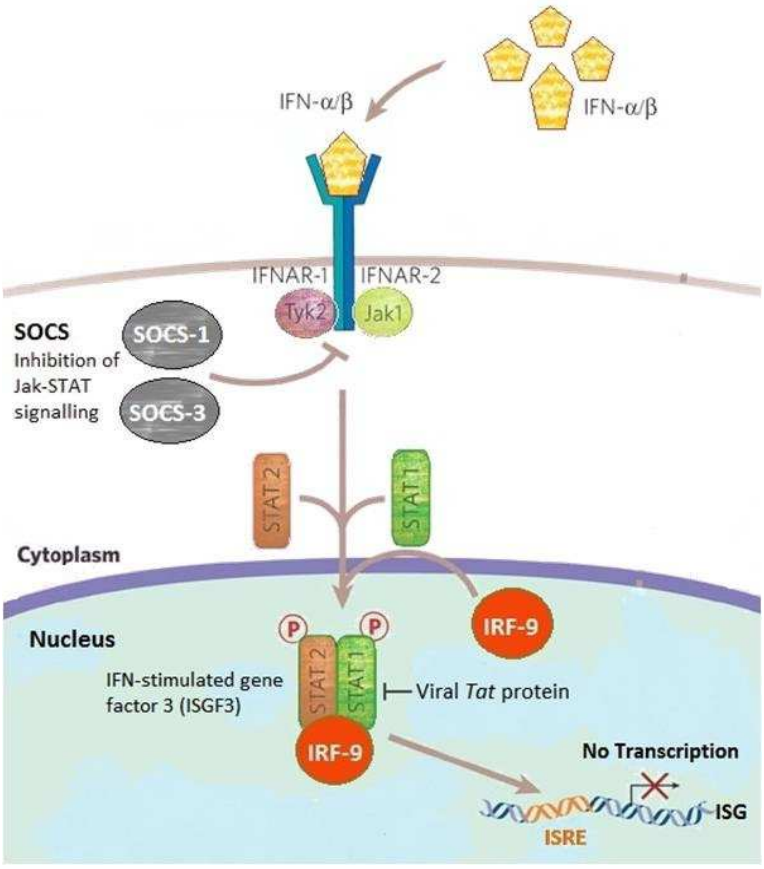
However, in early stage of HIV infection the sustained immune activation involves T cells stimulation and increased levels of phosphorylated STAT proteins along with partial inability of SOCS-1 and 3, inhibiting the JAK/STAT pathway. But, in later stage, the severe infection prevents the JAK/STAT activation, responsible for cytokine signaling mediated cell proliferation and differentiation of $CD4^+$ cells. Thus, HIV infection interfere the SOCS-1/3 expression driving immune activation. Also, at later stage the sustained immune activation disrupts lymphoid system and favors HIV-1 replication (Miller and Schlaepfer, 2011). Interestingly, the *in-vivo* and *in-vitro* experiments have also validated that SOCS-3 allows HIV-1 to evade innate immunity. The SOCS-3 expression attenuates the response of macrophages to IFN-β and thus preventing the HIV-1 replication from inhibitory effect of IFN-β (Yoshimura et al., 1995). Thus, it has been suggested a relation between high levels of SOCS-1/SOCS-3 expression and defects in innate immunity and adaptive Th1 responses, that is redirected in the AIDS patients with the loss of Th1 immune competence (Yadav et al., 2009). Therefore, it suggests that increased understanding of HIV-1 interaction with host protein could improve the therapeutic and prevention strategies to combat HIV/AIDS. However, efforts have been begun to target specific host mechanisms that are essential for HIV-1 replication but not for host cell itself.

Conclusions & Future Prospective

Above discussion has demonstrated the aberrant SOCS expression as a sponsor into shaping and overall presentation of viral disease. However, in recent research a cell signalling hormone called interleukin-7 (IL-7) which reinvigorates the immune response to chronic viral infection without too much collateral tissue damage, has been interestingly reported to cause suppression of SOCS gene expression at molecular level. In an overwhelming infection, SOCS-3 expression causes the immune system to slam on brakes too early and allow the infection to persist longer. But switching off the SOCS-3 gene by IL-7 has boosted the immune system to completely overcome and eliminate the infection. Since, SOCS proteins have a regulatory interference in the polarization of $CD4^+$ T cells into Th1, Th2, Th17 and T regulatory cell lineages, therefore, turning off the SOCS-3 expression can allow the immune system to boost the number of virus-specific T cells and have an immune response good enough to eliminate the virus without any autoimmune response. This remarkable finding has provided an excellent platform for new paradigm to develop the advanced therapies that could boost host immune response to fight disease, instead of targeting the disease only. It could help to develop new drugs that target SOCS-3 and turn it off for very short, defined periods of time to reinvigorate the T cells, allowing them to regroup to fight against infection. Hence SOCS expression can be a novel target to be regulated in viral diseases including AIDS in order to counteract immune activation.

Figure 1

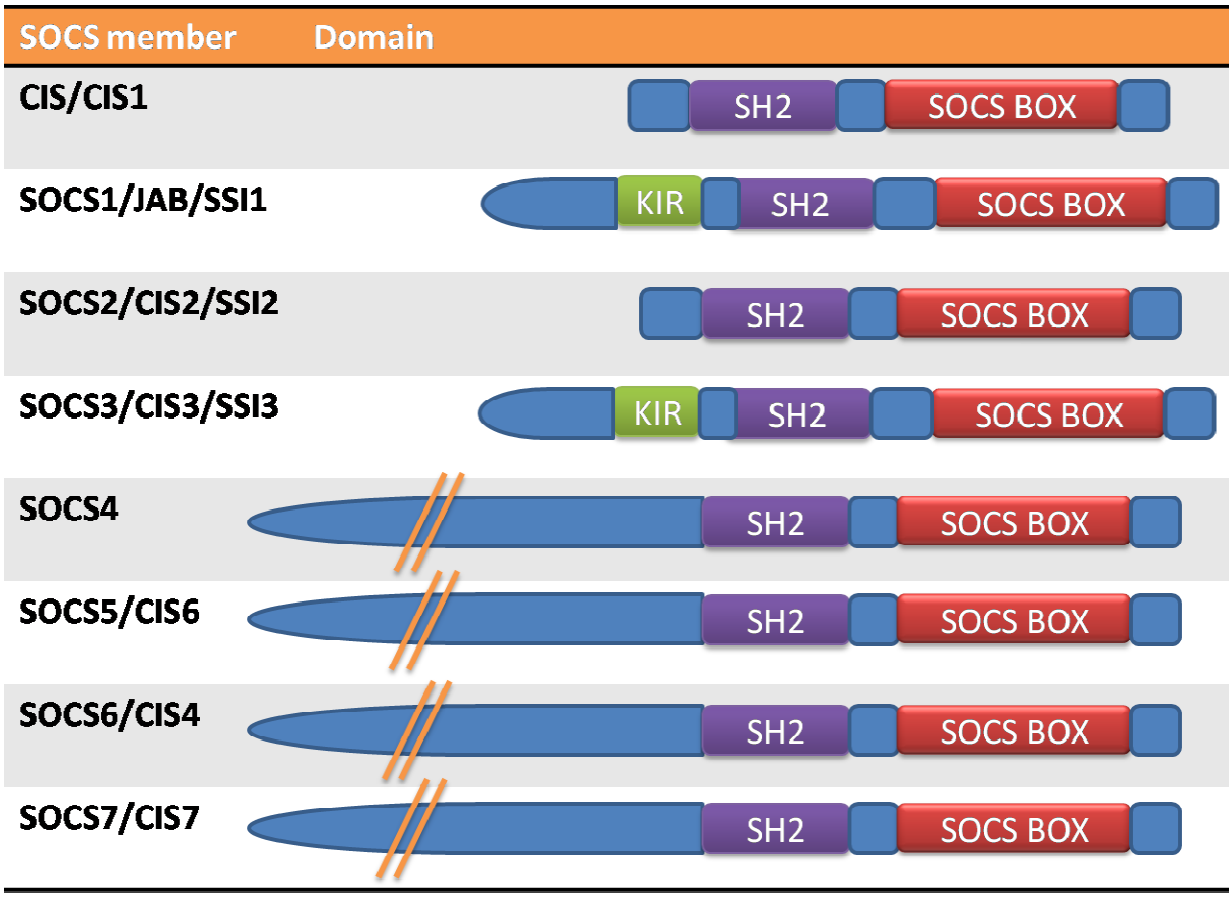
SOCS-1 & SOCS-3 mediated suppression of cytokine signaling and inhibitory effect of viral Tat protein on STAT dimerization to stop gene expression.



Following to stimulation of the cell surface receptor subunits IFN-α/β receptor 1 (IFNAR1) and IFNAR2 by Interferon (IFN), receptor-associated JAKs (JAK1 and TYK2) recruit and phosphorylate cytoplasmic STAT1 and STAT2. The STAT1-STAT2 heterodimer associates with IFN-regulatory factor 9 (IRF9) to form IFN-stimulated gene factor 3 (ISGF3), which enters the nucleus and binds IFN-stimulated regulatory elements (ISRE) in the promoters of hundreds of antiviral IFN-stimulated genes (ISGs) to induce their expression. ISGs have been shown to inhibit every stage of viral replication, from viral entry and uncoating to assembly and release, providing the host with formidable protection against viral infection.

Figure 2

The alternative names and domain structures of the SOCS family members. The Kinase Inhibitory Region (KIR) of SOCS-1 and SOCS-3 is marked green.



REFERENCES

1. Akhtar LN, Benveniste EN, Viral Exploitation of Host SOCS Protein Functions. J Virol., 85: 1912-1921, (2011).
2. Biggs BA, Hewish M, Kent S, Hayes K, Crowe SM, HIV-1 infection of human macrophages impairs phagocytosis and killing of *Toxoplasma gondii*. J Immunol., 154: 6132-6139, (1995).
3. Bode JG, IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J, 17: 488-490, (2003).

4. Cheng SM, Li JCB, Lin BSS et al., HIV-1 trans-activator protein induction of suppressor of cytokine signaling-2 contributes to dysregulation of IFN {gamma} signaling. Blood, 113: 5192-5201, (2009).
5. Danielle LK, and Douglas JH, SOCS: physiological suppressors of cytokine signaling. J Cell Sci., 113: 2813-2819, (2000).
6. Dwivedi LK, Shrivasatava M, Anti-HIV therapy: pipeline approaches and future directions. South Asian J Exp Biol., 1: 1-8, (2011).
7. Endo TA, Masuhara M, Yokochi M et al., A new protein containing an SH2 domain that inhibits JAK kinases. Nature, 387: 921–924, (1997).
8. Garrus JE, von Schwedler UK, Pornillos OW et al., Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. Cell, 107: 55-65, (2001).
9. Imada K, Leonard WJ, The Jak-STAT pathway. Mol Immunol., 37(1-2): 1-11, (2000).
10. Larsen L, Röpke C, Suppressors of cytokine signalling: SOCS. APMIS, 110: 833–44, (2002).
11. Liu R, Paxton WA, Choe S et al., Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell, 86: 367-377, (1996).
12. Ma X, Montaner LJ, Proinflammatory response and IL-12 expression in HIV-1 infection. J. Leukoc. Biol., 68: 383-390, (2000).
13. Miller RC, Schlaepfer E, HIV interferes with SOCS-1 and -3 expression levels driving immune activation. Article first published online: [21 FEB 2011] DOI: 10.1002/eji.201041198.
14. Moore JP, Coreceptors: implications for HIV pathogenesis and therapy. Science 276: 51-52, (1997).
15. Moutsopoulos NM, Vázquez N, Greenwell-Wild T et al., Regulation of the tonsil cytokine milieu favors HIV susceptibility. J Leukoc. Biol., 80: 1145-1155, (2006).
16. Murphy KM, Ouyang W, Farrar JD et al., Signaling and transcription in T helper development, Annu Rev Immunol., 18: 451-494, (2000).
17. Naka T, Narazaki M, Hirate M et al., Structure and function of a new STAT induced STAT inhibitor. Nature, 387: 924–929, (1997).
18. Platanias LC, Mechanisms of type-I- and type-II-interferon-mediated signaling. Nat Rev Immunol., 5: 375-386, (2005).
19. Pothlichet J, Chignard M, and 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. J Immunol., 180: 2034-2038, (2008).
20. Ram PA, Waxman DJ, SOCS/CIS protein inhibition of growth hormone-stimulated STAT5 signaling by multiple mechanisms. J Biol Chem., 274: 35553–35561, (1999).
21. Sakamoto H, Yasukawa H, Masuhara M et al., A Janus kinase inhibitor, JAB, is an interferon-gamma-induciible gene and confers resistance to interferons. Blood, 92: 1668–1676, (1998).
22. Samson M, Libert F, Doranz BJ et al., Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature, 382: 722-725, (1996).
23. Samuel CE, Antiviral actions of interferons. Clin Microbiol Rev., 14: 778-809, (2001).
24. Schroder K, Hertzog PJ, Ravasi T, Hume DA, Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol, 75: 163-189, (2004).
25. Song MM, 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).
26. Sporri B, Kovanen PE, Sasaki A, Yoshimura A, Leonard WJ,

JAB/SOCS1/SSI-1 is an interleukin-2-induced inhibitor of IL-2 signaling. Blood, 97: 221–226, (2001).
27. Starr R, Willson TA, Viney EM et al., A family of cytokine- inducible inhibitors of signaling. Nature, 387: 917–921, (1997).
28. Stoiber D, Kovarik P, Cohney S, Johnston JA, Steinlein P, Decker T, 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. J Immunol, 163: 2640-2647, (1999).
29. Sui B, Bamba D, Weng K et al., The use of Random Homozygous Gene Perturbation to identify novel host-oriented targets for influenza. Virology, 387: 473-481, (2009).
30. Tomic S, Chughtai N and Ali S, SOCS-1, -2, -3: Selective targets and functions downstream of the prolactin receptor. Mol Cell Endocrinol, 158: 45-54, (1999).
31. Vázquez N, Greenwell-Wild T, Rekka S, Orenstein JM, Wahl SM, *Mycobacterium avium*-induced SOCS contributes to resistance to IFN-γ-mediated mycobactericidal activity in human macrophages. J Leukoc Biol, 80(5): 1136-1144, (2006).
32. Wheeler J, McHale M, Jackson V, Penny M, Assessing theoretical risk and benefit suggested by genetic association studies of CCR5: experience in a drug development programme for maraviroc. Antivir Ther, 12: 233-245, (2007).
33. Yadav A, Fitzgerald P, Sajadi MM 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(1): 126-133, (2009).
34. Yoshimura A, Ohkubo T, Kiguchi T et al., A novel cytokine- induced gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. EMBO J, 14: 2816–2826, (1995).