Computer simulation of the role of SOCS family protein in helper T cell differentiation

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Abstract

The T₃₄₁/T₃₄₂ balance determines the nature of an immune response, and particular cytokines, IL-4 and IL-12, determine the direction at the initial stage of activation through TCRs. To investigate how cytokine networks and related signaling pathways impact upon the T₃₄₁/T₃₄₂ balance, we have developed a computer model for the simulation of T₃₄ differentiation. The model includes the IL-4, IL-12 and IFN-γ signal transduction pathways, a positive and negative feedback mechanism for cytokine signaling and cytokine-induced negative regulators such as suppressors of cytokine signaling (SOCS)1, SOCS3 and SOCS5. In the present study, we propose a ‘T₃₄₀ model’, in which naive T cells differentiate neither into T₃₄₁ nor into T₃₄₂ states in unskewed cytokine conditions. The model was found to be consistent with experimental results in BALB/c mice. The results of in silico analysis in the condition with SOCS- and signal transducer and activator of transcription (STAT) family-deficient and transgenic states were well fitted to ex vivo experimental results for T₃₄₁ and T₃₄₂ differentiation profiles in the deficient and transgenic mice. The T₃₄₀ model suggested the possibility that dominant T₃₄₁ differentiation in STAT4/STAT6 double-deficient mice may be due to a positive feedback effect of initial IFN-γ production from T cells. The in silico assessment of beneficial effects of inhibitory drugs by simulation analysis with our T₃₄₀ model indicated that Janus kinase 3-specific inhibitors might be suitable candidates for the modification of T₃₄₂-dominant immune responses. Our results demonstrate that models for the simulation of signaling network, such as our T₃₄₀ model, are useful tools for the in silico evaluation of novel drug candidates.

Introduction

A complex interplay of genetic and environmental factors contributes to the onset and maintenance of allergic immune disorders such as atopic dermatitis, pollinosis and bronchial asthma (1). It is known that cytokines secreted from T₃₄ differentiate from naive CD4⁺ T cells (Th0) into Th₁ or Th₂ subtypes (2). T₃₁ are responsible for defense against infectious intracellular micro-organisms, while T₃₂ are responsible for defense against extracellular pathogens and the development of allergic immune reactions (2). The balance of T₃₁ and T₃₂ is an important factor in immune responses, and the direction of T₃₄ differentiation is determined by the cytokine environment at the site of initial antigenic activation. The key cytokines for T₃₁ and T₃₂ differentiation are IL-12 and IL-4, respectively (2, 3).

The suppressors of cytokine signaling (SOCS) family of proteins are negative regulators of cytokine signaling (4–6). SOCS are induced by the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signal transduction pathway and inhibit JAK kinase activity by binding to JAK or cytokine receptors. SOCS1 is considered to be a common negative regulator in the JAK/STAT signal transduction pathway (5–8). We have shown that SOCS3 is predominantly expressed in T₃₂, that transgenic mice show increased T₃₂ responses and that mice with a dominant negative mutant SOCS3 show decreased Th₂ responses, suggesting that SOCS3 expression is correlated with allergic disorders. Therefore, SOCS3 is considered to have an important role in regulating the onset and maintenance of the T₃₂ phenotype (9). In addition, we have also shown that SOCS5 is predominantly expressed in T₃₁, and that it binds to the IL-4Rs and inhibits IL-4 signaling, thereby playing an important role in the T₃₁/T₃₂ balance (10). Therefore, the SOCS3 and SOCS5 activities...
may reflect a mechanism of mutual negative regulation in T\(_h\)1 and T\(_h\)2 development under unpolarized conditions. However, the detailed mechanisms of SOCS protein expression induced by cytokine signaling remain unclear.

T\(_h\) differentiation is tightly regulated by the balance of IL-12- and IL-4-mediated signaling. Moreover, IFN-\(\gamma\) produced from differentiated T\(_h\)1 is the key cytokine that regulates the persistence of T\(_h\)1 polarization. Thus, we reasoned that it may be possible to predict T\(_h\) differentiation pattern from cytokine signaling activity induced by cytokines at the initial stage of antigen stimulation. We have previously established a computer model to simulate the JAK/STAT signal transduction pathway (11). In this study, we attempted to establish a novel computer model, termed the ‘T\(_h\)0 model’, that is capable to simulate the T\(_h\)1/T\(_h\)2 balance on the basis of cytokine signaling network systems and, subsequently, to evaluate how well our model matches experimental data. A particular interest of this study lies with the elucidation of negative feedback mechanisms in cytokine signaling networks, such as SOCS1, SOCS3 and SOCSS. The differentiation behavior in the T\(_h\)0 simulation model was consistent with experimental profiles in SOCS and STAT knockout mice as well as in transgenic mice of SOCS molecules. Therefore, our T\(_h\)0 model should be a useful tool to simulate signaling processes that impact the T\(_h\)1/T\(_h\)2 differentiation and to estimate the biological behavior of signaling molecules and the effects of inhibitor drugs. We performed a sample simulation for such an in silico evaluation, and found that JAK3 inhibition may be a good therapeutic approach to mitigate T\(_h\)2-dominant responses.

Methods

Computational model

Cytokines determine the development of either T\(_h\)1 or T\(_h\)2 at an initial stage of activation through the TCR. The signal transduction pathway of the TCR may be involved in T cell differentiation, but since it similarly activates both T\(_h\)1 and T\(_h\)2 differentiation, it is unlikely to contribute significantly to the difference between T\(_h\)1 and T\(_h\)2 development. Therefore, the TCR signal transduction pathway was not included in the model. IL-12 and IL-4 are responsible for the development of T\(_h\)1 and T\(_h\)2, respectively, and thus the current model consists of signal transduction pathways induced by cytokines (IL-12, IL-4 and IFN-\(\gamma\)), rather than those induced by the TCR. The main pathways induced by cytokines are the JAK/STAT signal transduction pathways (2), and we have previously shown that the JAK/STAT signal transduction pathway induced by IFN-\(\gamma\) can be successfully modeled and simulated (11).

The model shown in Fig. 1(A) is the base model for the JAK/STAT signal transduction pathway. For the T\(_h\)0 model, the base model was simplified to draw three signaling pathways in the same graph, as shown in Fig. 1(B). As described above, IL-12 and IL-4 are responsible for T\(_h\)1 and T\(_h\)2 differentiation, respectively, and since IFN-\(\gamma\) is the key cytokine produced in T\(_h\)1, and may influence differentiation, the signal transduction pathway activated by IFN-\(\gamma\) was also included.

JAK2 and Tyk2 bound to IL-12Rs are activated by dimerization of receptors induced by IL-12 binding. This results in phosphorylation of STAT4, and the activated STAT4 dimers are then translocated to the nucleus and operate as transcription factors (Fig. 1A and C). JAK1 and JAK3 bound to IL-4Rs activate STAT6, and JAK1 and JAK2 bound to the IFN-\(\gamma\)-R activate STAT1 (11). SOCS3 is induced by IL-4 and binds to IL-12R–JAK complexes, inhibiting IL-12 signaling (9). By contrast, SOCS5 is induced by IL-12 and inhibits IL-4 signaling by binding to the IL-4R (10). SOCS1 is induced by all three cytokines, binds to JAK proteins and inhibits all three signals (5–8) (Fig. 1C).

T-box-binding protein (T-bet) and GATA-binding protein 3 (GATA3) are important transcription factors for T\(_h\)1 and T\(_h\)2 differentiation, respectively (12–15), and are induced by the IL-4 and IL-12 pathways, respectively (12–15). These molecules are part of positive feedback loops, which involve the induction of IL-4 production by GATA3 (12) and of IFN-\(\gamma\) production by T-bet and STAT1 (12) in IL-4 and IL-12/IFN-\(\gamma\) signaling, respectively. The induction of T-bet by IFN-\(\gamma\) (16) constitutes another feedback loop for IFN-\(\gamma\) signaling. Moreover, there are other interactions that provide positive feedback for IL-4 signaling. One subunit of the IL-12R, the \(\beta_2\) subunit, is induced indirectly by STAT4 (17), and STAT4 is induced in the IL-12 pathway (18). In addition to the mutual inhibition of SOCS3 and SOCSS, GATA3 inhibits transcription of the \(\beta_2\) subunit of the IL-12R (19). All the above feedback loops and mutually inhibitory reactions were included in the model.

Since changes in mRNAs for SOCS3 and SOCSS were slower than for SOCS1 (our unpublished results), the parameters for transcription and breakdown of mRNA for SOCS3 and SOCSS were set at smaller values than for SOCS1. Moreover, SOCS5 expression was slower than SOCS3 (9, 10) and the production rate of mRNA for SOCS5 was set at a smaller value than for SOCS3. Since, upon activation, cytokines are also produced by other lymphocytes, the background concentrations of IL-4, IL-12 and IFN-\(\gamma\) were all set at 0.5 nM.

All chemical reactions, including transcription, translation and translation, were described by differential equations and solved mathematically using the Runge–Kutta–Gill method, as described previously (11, 20). Parameter values and initial concentrations were set to values similar to those reported for the simulation of the IFN-\(\gamma\) signaling pathway (11). The detailed chemical reactions and their associated parameter values are described in the Supplementary Data (available at International Immunology Online).

Preparation of naive CD4 T cells and T\(_h\) development

CD62L\(^{hi}\) CD4\(^{+}\) T cells were purified with MACS beads from BALB/c mice. T cells were activated with plate-bound anti-TCR (H57-596) mAb (30 \(\mu\)g ml\(^{-1}\)) and soluble anti-CD28 (PV-1) mAb (10 \(\mu\)g ml\(^{-1}\)). After 5 days, cells were harvested and re-stimulated with anti-TCR mAb for 6 h in the presence of 4 \(\mu\)M monensin (Sigma-Aldrich, St Louis, MO, USA). Intracellular cytokine staining was carried out with FITC-labeled anti-IFN-\(\gamma\) mAb (XMG1.2) and PE-labeled anti-IL-4 mAb (11B11).

Results

Time course of signal transduction

The binding of cytokines to their receptors induces receptor dimerization, phosphorylation of receptors, autophosphorylation
of receptor-bound JAK, phosphorylation of STAT, dimerization of STAT and translocation of STAT to the nucleus (11). The dimerized form of phosphorylated STAT then acts as active transcription factor. The additional IL-4-induced activation of the IL-4R is followed by phosphorylation of STAT6 (Fig. 2A and C). Activation levels of STAT6 reached a maximum after ~1 h (Fig. 2A). In the previous JAK/STAT model (11), STAT phosphorylation was decreased by the inhibitory action of induced SOCS1. Similarly, SOCS1 acts as a general inhibitor of Th differentiation (5–8), IL-4 induced the expression of SOCS1 and SOCS3 and SOCS3 inhibited STAT4 activation (Fig. 2D). IL-4 is known to induce the expression of GATA3 which regulates the ability to secrete relatively high amounts of IL-4 in secondary responses (Fig. 2P). Moreover, GATA3 negatively regulated the expression of the β2 subunit of IL-12R.

On the other hand, IL-12-activated JAK bound to IL-12R and STAT4 (Fig. 2E). A peak of STAT4 activation also showed at ~1 h after initial TCR stimulation (Fig. 2E). Since the expression of IL-12R β2 subunits and STAT4 is controlled by IL-12 (data not shown), STAT4 activation (Fig. 2E) persisted longer than STAT6 activation (compare Fig. 2A with E). IL-12 induced both SOCS1 and SOCS5 in anti-TCR-activated CD4 T cells, and the induced SOCS5 inhibited STAT6 activation (Fig. 2B). IL-12 directly acted on the IFN-γ promoter and the induced IFN-γ promoted T-bet expression (data not shown), subsequently leading to the acquisition of the ability to produce IFN-γ in secondary T cell activation (Fig. 2T).

Simultaneous addition of IL-4 and IL-12 activated both Th1- and Th2-related pathways, resulting in subsequent differentiation of both Th1 and Th2 (Fig. 2, right panel). Since IL-4 promotes relatively high expression levels of SOCS3 protein (compare Fig. 2L with K), the steady state of STAT4 activation was shortened in the comparison with that of IL-12 addition alone (compare Fig. 2F with E). This contributed to the inhibitory function of SOCS3 in IL-12R-mediated STAT4 activation.

Concentration dependency of cytokine production

The direction of Th1/Th2 differentiation is determined by the presence of cytokines, and notably IL-12 and IL-4, during the initial activation of naïve T cells. In order to investigate the actual effect of additional cytokines in Th1 and Th2 differentiation, the in silico behavior upon addition of varying
concentrations of IL-4 and IL-12 in a variety of combinations was simulated and compared with ex vivo experimental results. We calculated induced IL-4 (black bar) and IFN-γ (white bar) production in secondary stimulation, after a 2-day initial incubation in the presence of various combinations of IL-4 (vertical axis) and IL-12 (horizontal axis) (Fig. 3). Figure 4 shows the experimental results of IL-4 and IFN-γ production observed in BALB/c CD4+ T cells. Highly purified CD4+ naive T cells were stimulated with plate-bound anti-TCR mAb in the presence of various combinations of IL-4 (0–1000 pM) and IL-12 (0–100 pM). In our model, stimulated T cells showed the ability to secrete low levels of IFN-γ even without the addition of IL-12 and IL-4 (Fig. 3, left bottom). Under the same culture conditions, BALB/c T cells exhibited very few IL-4- and IFN-γ-producing cells (Fig. 4). IL-4 and IFN-γ production levels gradually increased, depending on the respective concentrations of IL-4 and IL-12 added to the induction culture (Figs 3 and 4). IFN-γ production decreased with increased concentrations of IL-4, but IL-4 production remained at the same level even after the addition of higher amounts of IL-12 (Figs 3 and 4).
and 4). The overall profile shifted to Th2 differentiation in both model (Fig. 3) and experiment (Fig. 4). These results demonstrated that our simulation model correctly represents Th1 and Th2 differentiation profile experimentally observed in BALB/c T cells.

Role of SOCS proteins

The SOCS family of proteins is considered to play an important role in Th1/Th2 differentiation. SOCS1 is known to inhibit a wide range of cytokine-mediated pathways, and is expressed in all types of Th. SOCS3 and SOCS5 are exclusively expressed in Th1 and Th2, respectively, and critically impact Th differentiation (9, 10). However, the precise molecular mechanism through which SOCS regulates the Th1/Th2 balance in physiological conditions has not been completely clarified. It is experimentally difficult to monitor the expression level of SOCS protein in Th differentiation condition, because multiple cytokines are secreted from T cells and mutually regulate their function. Further, to examine the SOCS function at a particular stage of CD4 T cell differentiation using knockout or transgenic animal is unlikely to be successful. In fact, in SOCS1 knockout mice, T cell development process is impaired in thymus. Therefore, it is very difficult to assess the effect of deficiency at a particular stage of the cells. Computer simulation allows us to assess the effect of SOCS protein deficiency in mature CD4 T cells. IL-4 and IFN-γ production was simulated in silico under virtual SOCS1, SOCS3 and SOCS5 knockout (+/−/−), heterozygous (+/−/+) and transgenic conditions. The heterozygous state was simulated by setting the transcription rate of mRNA for each SOCS protein at half value as normal condition, and the knockout state was simulated by setting this rate to zero. The transgenic state was simulated by adding virtual transcription factors which constantly produce mRNA for SOCS. As shown in Fig. 5, SOCS1 knockout conditions (Fig. 5, broken lines) showed the highest activation of STAT4 and STAT6 and the highest expression of SOCS5 and SOCS3. Virtual SOCS5 knockout mice (broken–dotted lines) exhibited very slight changes in STAT4 and STAT6 activation and IL-4 and IFN-γ production compared with normal virtual mice. SOCS3+/− (heterozygous) and SOCS3−/− (dotted line) showed slight increase of IFN-γ production (Fig. 5 and compare Fig. 6C with A), resulting in a shift towards Th1 side (Fig. 7A and C), consistent with the experimental observation that T cells from heterozygous SOCS3+/− mice secrete larger amounts of Th1 cytokines during secondary stimulation (9). Furthermore, in our simulation, SOCS deficiency did not significantly impact Th1/Th2 balance (Fig. 7B and D) or IL-4 production (Figs 5 and 6). This might be due to weaker expression levels of SOCS5 induced by IL-12 than the expression level of SOCS3 induced by IL-4, which was observed experimentally (our unpublished result). This in silico result is consistent with the experimental data that Th1/Th2 balance in SOCS5−/− mice is indistinguishable from that in normal mice (21). Under SOCS1-deficient conditions, the balance shifts towards the Th2 side (Fig. 6B). This is likely due to a higher level of SOCS3 protein, as compared with SOCS5, in this condition (Fig. 5). In contrast, the in silico result of SOCS1-deficient mice was inconsistent with the result of ex vivo experiments, which show Th1 dominance (8, 22).

Simulation of constitutive expression of SOCS3 and SOCS5 in T cells inhibited the IL-12 and IL-4 signaling pathways, thus leading to skewed Th2 and Th1 responses in secondary stimulation, respectively (Fig. 7E and F). These profiles are consistent with experimental results in transgenic mice which showed that SOCS3 and SOCS5 were expressed under the control of the lck promoter (9, 10). These results support the significance of our Th0 model to assess the role of the signaling regulator in an in silico evaluation.
Role of STAT family proteins

Since STAT proteins are key transcription factors in cytokine signaling, deficiency in STAT genes can attenuate the signaling pathway that is regulated by particular cytokines, resulting in a drastic effect on Th1 and Th2 differentiation. Both STAT4- and STAT6-deficient mice show clearly diminished Th1 and Th2 differentiation, respectively (23–25). However, the Th1 response still remains active in STAT4 and STAT6 double knockout mice (26, 27). To understand how IFN-γ production is induced in the double-deficient condition, we simulated IFN-γ and IL-4 production in the secondary response by setting the initial concentration of either STAT4 or STAT6 to zero. In the Th0 model, the double knockout condition of STAT4 and STAT6 exhibited detectable IFN-γ production, but no IL-4 production (26, 27). To understand how IFN-γ production is induced in the double-deficient condition, we simulated IFN-γ and IL-4 production in the secondary response by setting the initial concentration of either STAT4 or STAT6 to be zero. In the Th0 model, the double knockout condition of STAT4 and STAT6 exhibited detectable IFN-γ production, but no IL-4 production (Fig. 8C), although single knockout condition showed loss of either IFN-γ or IL-4 production (Fig. 8A and B). Our model suggested that initial IFN-γ production from CD4 T cells induced T-bet expression (Fig. 1C) and, thus, the autocrine feedback loop in IFN-γ production impacts upon the generation of Th1 in STAT4 and STAT6 double-deficient T cells. These results indicated that the Th0 model would be useful to predict the behavior in signal transduction pathways under particular conditions.

Simulation of disease states and a virtual evaluation of drug effects

The in silico evaluation of the biological effects of drug candidates in a well-characterized signaling pathway is a potentially very powerful application of simulation analysis. In what follows, we show that our Th0 model is a useful heuristics for the in silico screening of drugs that impact upon the Th1/Th2 differentiation process. An excess Th2 response is thought to facilitate the development and persistence of allergic disorders. There are several molecular mechanisms of an excess Th2 response, such as excess IL-4 and IL-13 production, excess expression of IL-4R and SOCS3 and reduced SOCS5 expression. Polymorphisms of the IL-4R and STAT6 genes have been reported to correlate with the exacerbation of allergic diseases (28). To establish a Th2-dominant response, the sensitivity against IL-4 in our Th0 model was increased by the enhancement of the expression level of IL-4Rs. Thus, the level of IL-4 expression was markedly increased, while IFN-γ...
production was mostly inhibited (Fig. 9B). To simulate the effect of drugs in the Th0 model, we chose a JAK3-specific hypothetical drug named as ICP, which is similar to the immunosuppressant CP-690,550 (29). The binding constants of ICP with the JAK family were set to those of CP-690,550 and binding constants with JAK1, JAK2 and JAK3 of 100, 20 and 1 nM, respectively, were used. To compare drug actions, a second hypothetical drug named as IA, that binds to all the JAK family proteins with the same binding constant (10 nM), was also tested. Both virtual drugs showed inhibition of excess IL-4 production (Fig. 9C and D). The non-specific JAK inhibitor, IA, showed inhibition of both IL-4 and IFN-γ production (Fig. 9C), whereas the JAK3-specific drug, ICP, showed less inhibitory effect on IFN-γ production (Fig. 9D). These simulations suggest that JAK3-specific drugs are likely to be better drug candidates for Th2-mediated disorders.

Discussion

We have developed a computer model for the simulation of Th differentiation that takes into account IL-4, IL-12 and IFN-γ signaling and is based on our earlier JAK/STAT signal transduction pathway model (11). The model simulates cytokine-based Th1 and Th2 differentiation, including transient signals and mutual inhibitory actions of SOCS1, SOCS3 and SOCS5 (Fig. 2). The cytokine production profile in our model (Fig. 3) was consistent with observed profiles in BALB/c T cells (Fig. 4). Importantly, these data demonstrated that our current mathematical model can simulate several aspects in Th1/Th2 differentiation.

Moreover, the behavior of the model under conditions simulating knockout (−/−), heterozygous (+/−) and transgenic SOCS1, SOCS3 and SOCS5 states (Figs 5–7) was partly supported by previous experimental results (9, 10, 21, 22). As shown in Fig. 5, SOCS1 knockout conditions (Fig. 5, broken lines) showed considerable impact on STAT activation and the expression of other SOCS family members. This would explain the appearance of several inflammatory reactions and lethality at an early age (22). Further, it suggests that SOCS1 regulates both Th1 and Th2 differentiation. Moreover, in the IFN-γ signaling pathway, SOCS1 is thought to regulate both transient (peak height and width) and steady state in IFN-γ-induced STAT1 activation (11), and our Th0 model clearly suggests a similar profile in STAT1 activation. Although the inhibitory function of SOCS1 equally acts on the IL-4 or IL-12 signaling pathways, the model suggested that IL-4 signals, rather than IL-12 signals, were increased in the absence of SOCS1. In our current Th0 model, the differential effect of SOCS1 in IL-4- and
IL-12-mediated Th development is explained by the fact that SOCS1 simply amplifies SOCS3 and SOCS5 activity. Therefore, the inhibitory activity of SOCS3 exceeds that of SOCS5, thus our model addresses Th2 dominancy.

Under either STAT4- or STAT6-deficient conditions, the cytokine expression profile was consistent with reported experimental results (23–25). In previous reports, STAT4/STAT6 double knockout T cells were found to retain the potential to differentiate into Th1 (26, 27), and the Th0 model suggested that initial IFN-γ production in the double knockout T cells may promote the positive feedback loop in the IFN-γ signal transduction pathway. Activation of STAT1 by IFN-γ initiates T-bet expression, which plays a critical role in the acquisition of the ability to produce IFN-γ in Th1 differentiation. Therefore, it is

**Fig. 7.** Calculated concentration dependency of cytokine production under SOCS+/+ (A, B), SOCS−/− (C, D), transgenic states (E, F) of SOCS3 (A, C, E) and SOCS5 (B, D, F). Graphs are normalized to the maximum of each graph.
likely that the positive feedback loop in IFN-γ-mediated signaling is a possible mechanism to promote Th1 response in STAT4/STAT6 double knockout mice.

The unpolarized condition shown in Fig. 1 contains mutual inhibitory interactions and positive feedback mechanisms for both the IL-4 and IL-12/IFN-γ pathways. Although some systems with such mutual inhibitory interactions and positive feedback loops show exclusive output, both simulation results (Fig. 3) and experimental results (Fig. 4) did not show exclusive cytokine production. The reasons for this are as follows. First, the unpolarized condition has common negative regulators, such as SOCS1. Since the output size of the larger input signal is inhibited to the medium size by SOCS1, it fails to inhibit the opposite side of the signal pathway completely. Second, positive feedback loops for both IL-4 and IFN-γ consist of several time-consuming steps. Since cytokine production is only slowly induced in the unpolarized condition, the output of each pathway is insufficient to suppress the opposite pathway. Third, the kinetics of inhibitory actions by SOCS3 and SOCS5 is likely to be distinctive, and therefore it is unlikely to inhibit the opposite signal completely (see Fig. 2).

Based on the combination of these factors, the unpolarized condition did not reach the state of exclusive cytokine production. Since the activities of the mutual inhibitory interactions by SOCS3 and SOCS5 are different, the cytokine production profile in our model was slightly shifted towards the Th2 side (Fig. 3). These results clearly demonstrate that the current model is able to simulate Th1/Th2 differentiation that occurs in the unpolarized condition of the BALB model system. Although the mass reactions simulated in the Th0 model do not reveal the reactions of each molecule in a single cell, they appear to monitor the average potential to produce either IFN-γ or IL-4 in the entire cell population. At present, our model cannot predict fine details in each signaling cascade at the single-cell level. Towards this end, various other factors, including notably the stability of receptors and signaling molecules, need to be further investigated.

In conclusion, our simulation model effectively reproduces the actual Th1/Th2 average balance in the unpolarized condition of the BALB/c strain and, further, our model allows us to monitor the role of SOCS and STAT family proteins in Th1 differentiation under conditions that cannot be realized experimentally. From the sample simulation (Fig. 9), the model was also shown to be useful for investigations of the molecular mechanisms of disease and the evaluation of drug candidates in silico. Our model can simulate the cytokine balance regulating Th1/Th2 differentiation and is thus a good starting point for future efforts to simulate complex immune responses in T cells.

**Fig. 8.** Calculated concentration dependency of cytokine production under (A) STAT4 knockout, (B) STAT6 knockout and (C) STAT4/STAT6 double knockout conditions. Graphs are normalized to the maximum of each graph.
Supplementary data

Supplementary data are available at International Immunology Online.

Abbreviations

GATA3  GATA-binding protein 3
JAK    Janus kinase
SOCS   suppressors of cytokine signaling
STAT   signal transducer and activator of transcription
T-bet  T-box-binding protein

References


