Innate Immunity in Pluripotent Human Cells

ATTENUATED RESPONSE TO INTERFERON-β

Received for publication, November 10, 2012, and in revised form, April 18, 2013. Published, JBC Papers in Press, April 18, 2013, DOI 10.1074/jbc.M112.435461

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Background: Interferon signaling is an important component of innate immunity.

Results: Human pluripotent cells fail to respond to interferon at least partly due to the high expression of an inhibitor of STAT1 phosphorylation, SOCS1.

Conclusion: Pluripotent cells may be poised for the activation of interferon response upon differentiation.

Significance: Pluripotency is associated with shutoff of a specific signal transduction pathway.

Type I interferon (IFN-α/β) binds to cell surface receptors IFNAR1 and IFNAR2 and triggers a signaling cascade that leads to the transcription of hundreds of IFN-stimulated genes. This response is a crucial component in innate immunity in that it establishes an “antiviral state” in cells and protects them against further damage. Previous work demonstrated that, compared with their differentiated counterparts, pluripotent human cells have a much weaker response to cytoplasmic double-stranded RNA (dsRNA) and are only able to produce a minimal amount of IFN-β. We show here that human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) also exhibit an attenuated response to IFN-β. Even though all known type I IFN signaling components are expressed in these cells, STAT1 phosphorylation is greatly diminished upon IFN-β treatment. This attenuated response correlates with a high expression of suppressor of cytokine signaling 1 (SOCS1). Upon differentiation of hESCs into trophoblasts, cells acquire the ability to respond to IFN-β, and this is accompanied by a significant induction of STAT1 phosphorylation as well as a decrease in SOCS1 expression. Furthermore, SOCS1 knockdown in hiPSCs enhances their ability to respond to IFN-β. Taken together, our results suggest that an attenuated cellular response to type I IFNs may be a general feature of pluripotent human cells and that this is associated with high expression of SOCS1.

Type I IFN is induced by cytoplasmic dsRNA, viruses, and other intracellular infections (1, 2). Once secreted, it acts in an autocrine or paracrine manner and triggers tyrosine phosphorylation and activation of members of the Janus kinase (JAK) family of cytoplasmic tyrosine kinases, JAK1, and tyrosine kinase 2 (TYK2). Phosphorylated JAKs then activate the phosphorylation of signal transducers and activator of transcription 1 (STAT1) and STAT2. Phosphorylated STAT1 and STAT2 undergo dimerization and association with IFN-regulatory factor 9 (IRF9) to form IFN-stimulated gene factor 3 (ISGF3). These complexes then translocate to the nucleus and bind IFN-stimulated response elements (ISREs) in DNA to activate the transcription of hundreds of IFN-stimulated genes (ISGs), which mediate various important biological processes in the cell (for reviews, see Refs. 2, 3).

Sustained action of IFN-mediated signaling can be harmful to cells. Accordingly, a number of negative regulators have been identified to prevent the overreaction of cells to these cytokines. SH2-containing phosphatase-1 (SHP-1) can dephosphorylate JAKs, thus terminating type I IFN signaling (4, 5). Other JAK phosphatases have also been reported, including tyrosine phosphatase 1B (PTP1B) (6), T cell protein-tyrosine phosphatase (TCPTP) (7), and CD45 (8). Protein inhibitors of activated STATs (PIAS) are another class of inhibitors for IFN-mediated signaling and can bind directly to STATs and inhibit their function (9, 10). More importantly, recent studies suggest that the protein family of suppressor of cytokine signaling (SOCS) includes potent negative regulators for type I IFN signaling (for reviews, see Refs. 11–14). The SOCS family is composed of eight members: cytokine-inducible SRC homology 2 (SH2) domain-containing protein (CIS) and SOCS1–SOCS7. These associate with phosphorylated tyrosine residues on activated JAKs and/or cytokine receptor subunits through a central SH2 domain. A C-terminal SOCS box then interacts with components of the ubiquitin ligase machinery and mediates proteasomal degradation of associated proteins, causing the termination of downstream signaling events (15). SOCS proteins are generally expressed at low levels in unstimulated cells. Upon stimulation with cytokines, they are rapidly induced and function as negative regulators of JAK/STAT signaling, thus creating a classic negative feedback loop. Among all the members of the SOCS protein family, SOCS1 and SOCS3 are the most important in regulating cellular innate and adaptive immune responses, with SOCS1 being especially effective for the inhibition of STAT1 phosphorylation (16, 17).

*This work was supported by an Established Investigator award from the State of Connecticut under the Connecticut Stem Cell Research Grants Program (to G. G. C.).

This article contains supplemental Table S1.

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2 The abbreviations used are: ISG, IFN-stimulated gene; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; qPCR, quantitative PCR; SH2, SRC homology 2; SOCS, suppressor of cytokine signaling.
In differentiated mammalian cells, the introduction of dsRNA into the cytoplasm usually leads to a potent antiviral response resulting in the rapid induction of IFN-β. However, in mouse oocytes and early embryos long dsRNA induces efficient RNAi but does not trigger apoptosis (18, 19), suggesting a defect in cytoplasmic dsRNA response. We showed recently that human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) lack this response because of the systematic down-regulation of a number of genes involved in cytoplasmic responses to dsRNA (20). In the current study, we extend that work by showing that these human pluripotent cells also do not respond to IFN-β. This is partly due to a modest down-regulation of some of the signaling molecules in the type 1 IFN response pathway in these cells. More importantly, STAT1 phosphorylation upon IFN-β treatment is almost completely absent, and this defect correlates with a high basal level of SOCS1 expression. Consistent with this hypothesis, knockdown of SOCS1 in hiPSCs leads to a stronger response to IFN-β.

EXPERIMENTAL PROCEDURES

Antibodies and siRNAs—Primary antibodies against MDA5, RIG-I, JAK1, tyrosine kinase 2, STAT1, phospho-STAT1 (Y701), STAT2, STAT3, STAT1 (Y705) were from Cell Signaling Technology; those for IFNAR1, IFNAR2, IFN-regulatory factor 9, MX1, and actin were from Abcam. Phospho-STAT2 (Tyr689) and Sox2 antibody were from Millipore, and SOCS1 antibody was from Santa Cruz Biotechnology. Horseradish peroxidase-conjugated goat anti-rabbit IgG (Millipore) and sheep anti-mouse IgG (GE Healthcare) were used as secondary antibodies for immunoblots. Silencer® Select Pre-designed siRNAs against different sequences within socs1, and the negative control siRNA were purchased from Invitrogen.

Cell Lines and Trophoblast Differentiation—IMR-90, MCF-7, HEK293, and HeLa cells were cultured in DMEM supplemented with 10% FBS. IMR-90 cells between passages 2 and 6 were used in this study. iPSC(IMR-90) were generated from IMR-90 precursor cells and were verified at the University of Connecticut Stem Cell Core (21). They were confirmed positive for Tra-1-81, Tra-1-60, SSEA-3 and SSEA-4 by immunofluorescence and teratoma formation (22). Human embryonic stem cell lines H1, H9, and CT2, along with iPS(IMR-90) cells were maintained on plates coated with Matrigel® (BD Biosciences) with defined mTeSR™1 medium (StemCell Technologies). Pluripotent human cells were evaluated for Oct3/4 expression every 3–4 weeks, and cells were passaged every 6–7 days. For trophoblast differentiation, H9 cells were treated with 100 ng/ml BMP4 (R&D Systems) for 7 days in mTeSR™1 medium, as described previously (23, 24). All cells were maintained at 37 °C in 5% CO₂.

RT-PCR and q-PCR Analysis—Total RNAs were isolated using TRIzol® reagent (Invitrogen). After treatment with DNase I (Invitrogen; DNA-free™ kit), cDNA was made with SuperScript III reverse transcriptase (Invitrogen) with random hexamers. The obtained cDNAs were used for subsequent PCR and qPCR analysis. iQ™ SYBR® Green Supermix (Bio-Rad) was used for qPCRs to detect levels of individual mRNAs. The qPCRs were conducted using an iQ5 real-time PCR detection system. The primers used for RT-PCR and RT-qPCR analysis are listed in supplemental Table S1.

SDS-PAGE and Immunoblot Analysis—Cells were washed twice with ice-cold PBS, and whole cell extracts were obtained using radioimmuneprecipitation assay lysis buffer (1 × PBS, 1% Nonidet P-40, 0.1% SDS, 1% deoxycholate; protease and phosphatase inhibitors from Roche Applied Science were added before use). Protein concentrations were determined using the Bio-Rad DC Assay Kit. Immunoblots from SDS-PAGE gels were performed using various primary antibodies and horseradish peroxidase-conjugated and species appropriate IgG secondary antibodies diluted in blocking buffer. Blots were developed using ECL reagents (GE Healthcare) on a Kodak Image Station 4000MM Pro.

siRNA Knockdown—SOCS1 knockdown in iPSC(IMR-90) cells was carried out using Lipofectamine 2000 (Invitrogen) as described previously (25). A second transfection with siRNAs was carried out 36 h after the initial transfection, following the same procedure. 60 h after the initial knockdown, fresh mTeSR™1 medium with or without 1000 IU/ml IFN-β was...
added to the cells, and RNA and protein samples were obtained from the cells 6 h after IFN-β treatment.

Statistical Analysis—Data from individual experiments are represented as means ± S.E. unless otherwise stated. Statistical comparison of groups was performed using a two-tailed Student’s t test or analysis of variance with appropriate tests for equal variances. Statistical significance was defined and indicated as **, p < 0.01; ***, p < 0.001.

RESULTS

Human Pluripotent Cells Have an Attenuated Response to IFN-β—Human diploid fibroblast IMR-90 cells and iP(iPS(IMR-90)) cells were treated with 1000 IU/ml IFN-β for various times, and the expression of several ISGs was analyzed by RT-PCR. IFN-β initiated a rapid induction of ISG expression in IMR-90 cells within 1 h, with much higher expression levels of these genes observed after 6 h (Fig. 1A, lanes 5–8). In comparison, the response of iP(iPS(IMR-90)) cells to IFN-β was greatly attenuated, with little elevation of ISG expression even at 6 h after IFN-β treatment (Fig. 1A, lanes 1–4). To further quantify the difference in the response of IMR-90 and iP(iPS(IMR-90)) cells to IFN-β, samples were subjected to RT-qPCR analysis (Fig. 1B). Whereas the treatment of IFN-β led to a 30 to a >3000-fold increase in ISG expression in IMR-90 cells, the induction of these genes in iP(iPS(IMR-90)) cells was <4-fold. Immunoblot analysis also showed that 6 h of IFN-β treatment led to a significant increase in the levels of protein expression of the ISGs MX1, MDA5, and RIG-I in IMR-90 cells (Fig. 1C, lane 8), whereas the response in iP(iPS(IMR-90)) cells was diminished (Fig. 1C, lanes 1–4).

In addition, hESCs (H9) and trophoblasts derived from them using a 7-day BMP4 differentiation method (23, 24) were also examined for their responses to IFN-β. Similar to the previous observations, IFN-β treatment led to a noticeable induction of ISG expression in H9 cells within 1 h (Fig. 2A, lanes 5–8). However, in H9 cells the induction of ISG expression was only observed at 6 h after IFN-β treatment and was strongly attenuated compared with the response in H9+BMP4 cells (Fig. 2A, lanes 1–4). IFN-β treatment for 6 h led to a 50- to >2000-fold induction of ISG expression in

![Figure 2](image2.png)

**FIGURE 2.** Human ES cells have an attenuated response to IFN-β. A, H9 and H9+BMP4 cells were treated with 1000 IU/ml IFN-β for 15 min, 1 h, and 6 h, and the expression of several ISGs was examined using RT-PCR. B, differences in the induction of ISG expression at 6 h after IFN-β treatment were further quantitated by RT-qPCR. The mRNA expression levels were normalized to actin and gapdh, with the gene expression in H9+BMP4 cells without IFN-β treatment set as 1. C, protein expression of MX1, MDA5, and RIG-I after 6-h IFN-β treatment was analyzed by immunoblotting.

![Figure 3](image3.png)

**FIGURE 3.** Expression of IFN signaling molecules in human pluripotent cells. A, lower expression of key type I IFN signaling pathway components in iP(iPS(IMR-90)) cells, analyzed by RT-qPCR. B, lower expression of key type I IFN signaling pathway components in H9 cells analyzed by RT-qPCR. The mRNA expression levels were normalized to actin and gapdh. **, p < 0.01; ***, p < 0.001. Error bars, S.E. C, Protein expression of IFN-β signaling molecules in pluripotent and differentiated human cells.
TABLE 1
Relative expression of genes involved in cellular responses to type I IFNs

| Gene     | RPKM H9 | RPKM HeLa | Function                      |
|----------|---------|-----------|-------------------------------|
| ifnar1   | 0.93    | 6.25      | IFN-β signaling pathway       |
| ifnar2   | 1.32    | 4.32      | IFN-β signaling pathway       |
| jak1     | 2.30    | 7.39      | IFN-β signaling pathway       |
| tyk2     | 4.13    | 14.09     | IFN-β signaling pathway       |
| stat1    | 8.42    | 8.60      | IFN-β signaling pathway       |
| stat2    | 5.43    | 5.78      | IFN-β signaling pathway       |
| irf9     | 2.21    | 3.25      | IFN-β signaling pathway       |
| ptpn6    | 6.68    | 3.55      | JAK phosphatase               |
| ptpn1    | 10.38   | 13.18     | JAK phosphatase               |
| ptprc    | 0       | 0         | JAK phosphatase               |
| ptprc1   | 4.2     | 7.98      | JAK phosphatase               |
| ddx1p1   | 4.75    | 8.81      | Protein inhibitor of activated STATs |
| pias4    | 17.39   | 9.67      | Protein inhibitor of activated STATs |
| sox1     | 15.85   | 0.00      | Suppressor of cytokine signaling |
| sox3     | 7.29    | 9.48      | Suppressor of cytokine signaling |
| nanog    | 21.83   | 0.05      | Stem cell marker              |
| sox2     | 70.59   | 0.00      | Stem cell marker              |
| lin28    | 285.03  | 0.03      | Stem cell marker              |
| oct3/4   | 559.51  | 0.21      | Stem cell marker              |
| gapdh    | 1727.11 | 1230.46   | Housekeeping gene             |

FIGURE 4. Examination of STAT1 phosphorylation state in pluripotent human cells. A, STAT1 phosphorylation induced by IFN-β is not observed in iP(S)(IMR-90) cells. Immunoblot analysis of phospho-STAT1(Tyr701) levels in IMR-90 and iP(S)(IMR-90) cells was performed at different time points after IFN-β treatment. B, immunoblot analysis of phospho-STAT1(Tyr701), phospho-STAT2(Tyr517), and phospho-STAT3(Tyr705) levels in pluripotent and differentiated human cells was done at 6 h after IFN-β treatment.

H9+BMP4 cells, but <10-fold in H9 cells (Fig. 2B). MX1, MDA5, and RIG-I protein levels were also significantly induced after a 6-h IFN-β treatment in H9+BMP4 cells, but not in H9 cells (Fig. 2C).

Human Pluripotent Cells Express Signaling Molecules in the Type I IFN Response Pathway—The expression of IFN signaling components in differentiated and pluripotent human cells were next analyzed using RT-qPCR. The results showed that iP(S)(IMR-90) cells expressed ifnar1, jak1, and tyk2 at somewhat lower levels compared with IMR-90 cells (Fig. 3A). The expression of these genes, along with ifnar2, stat2, and irf9, is also lower in H9 cells than in H9+BMP4 cells (Fig. 3B). In agreement with these observations, deep sequencing analysis using H9 and HeLa cells (26) also suggested that ifnar1, ifnar2, jak1, and tyk2 were expressed at modestly lower levels in H9 cells than in HeLa cells (Table 1). However, immunoblot analysis using antibodies (including multiple antibodies against the same target) for these signaling molecules consistently revealed less striking differences in their expression levels between differentiated and pluripotent human cells (Fig. 3C and data not shown). Taken together, these data suggest a slight diminution in expression levels of some of these factors in pluripotent cells, but not enough to account for the almost total lack of response to IFN-β.

STAT1 Phosphorylation Is Defective in Human Pluripotent Cells—Phosphorylation of the STAT1 transactivating domain is a crucial step in cellular responses to type I IFNs. In IMR-90 cells, a significant induction of STAT1 phosphorylation occurred as early as 15 min after IFN-β treatment, reached its peak level at 1 h, and receded to a lower level after a 6-h IFN-β...
treatment (Fig. 4A, lanes 5–8). Strikingly, STAT1 phosphorylation was not observed in iPS(IMR-90) cells at any of the time points examined (Fig. 4A, lanes 1–4). Similarly, IFN-β treatment in H9+BMP4 cells also led to a strong activation of STAT1 phosphorylation at 6 h, which was absent in H9 cells under the same conditions (Fig. 4B). Importantly, STAT2 phosphorylation upon IFN-β treatment did not appear to be significantly induced in either pluripotent or differentiated cells (Fig. 4B). STAT3 phosphorylation, which induces the formation of STAT3-STAT3 homodimers or STAT1-STAT3 and STAT2-STAT3 heterodimers that bind to IFN-γ-activated site elements on DNA (3), was likewise not strongly induced in either pluripotent or differentiated cells after IFN-β treatment (Fig. 4B).

To determine whether the defect in STAT1 phosphorylation and the resulting attenuated response to type I IFNs reflect a general feature of human pluripotent cells, several other cell lines were examined for their response to IFN-β. Whereas STAT1 was strongly phosphorylated in MCF-7 and HEK293 cells after IFN-β treatment (Fig. 5A, lanes 7–10), no apparent induction of STAT1 protein expression was observed in H9, H1, and CT2 cells. The levels of gene expression were normalized to *gapdh*. ***, *p* < 0.001. Error bars, S.E.
Human Pluripotent Cells Have an Elevated Expression of SOCS1—The above results show that human pluripotent cells are defective in STAT1 phosphorylation in response to type I IFNs. Whereas some of the upstream signaling molecules in the IFN response pathway are expressed at modestly lower levels compared with differentiated cells, these reduced levels do not appear to be sufficient to account for the strikingly attenuated response to type I IFNs in these cells. To further elucidate the cause of the defect in STAT1 phosphorylation in human pluripotent cells, we compared the expression levels of several negative regulators of type I IFN signaling between H9 and HeLa cells using deep sequencing analysis (26). Interestingly, SOCS1, a potent inhibitor of STAT1 phosphorylation, is expressed at a much higher level in H9 cells compared with HeLa cells (Table 1). The expression level of socs1 in differentiated and pluripotent human cells was further determined by RT-PCR. Without IFN-β treatment, socs1 was expressed at a low basal level in IMR-90 cells, whereas iPS(IMR-90) cells exhibited a significantly higher expression of socs1 (Fig. 6A, lanes 1 and 3). In accordance with its involvement in a negative feedback loop to attenuate cytokine signaling, the expression of socs1 was induced in IMR-90 cells after a 6-h IFN-β treatment. However, its expression remained almost unchanged in iPS(IMR-90) cells (Fig. 6A, lanes 2 and 4). RT-qPCR analysis confirmed this observation (Fig. 6B). SOCS1 protein expression was also elevated in iPS(IMR-90) compared with IMR-90 cells (Fig. 6C). H9 and H9+BMP4 cells showed a similar pattern of socs1 expression before and after 6-h IFN-β treatment (Fig. 6D). In addition, H1 and CT2 cells also expressed higher levels of socs1 than the differentiated human HEK293 and HeLa cells (Fig. 6, E and F). A higher protein expression of SOCS1 was further observed in H9, H1, and CT2 cells compared with H9+BMP4 and MCF-7 cells (Fig. 6G).

Reduction of SOCS1 Expression Leads to a Stronger Response to IFN-β in Human Induced Pluripotent Stem Cells—To address the significance of the high expression of SOCS1 observed in human pluripotent cells, iPS(IMR-90) cells were treated with two siRNAs targeting different sequences in the socs1 gene. The knockdown efficiency for socs1 was examined by RT-PCR and RT-qPCR. SOCS1 siRNA-1- and siRNA-2-treated iPS(IMR-90) cells demonstrated a 50% and 70% decrease in socs1 expression, respectively, compared with the negative control siRNA-treated cells (Fig. 7, A and B). The reduction of SOCS1 protein expression after knockdown was further confirmed by immunoblot analysis (Fig. 7C).

Upon 6-h IFN-β treatment, SOCS1 siRNA-1- and siRNA-2-treated iPS(IMR-90) cells showed significantly higher STAT1 phosphorylation levels than the negative control siRNA-treated cells, whereas STAT2 and STAT3 phosphorylation remained largely unaffected (Fig. 8A). At the same time, the iPS(IMR-90) cells with reduced socs1 expression also exhibited an elevated induction of ISG expression upon IFN-β treatment (Fig. 8B). This observation was further quantitated by RT-qPCR (Fig. 8C). Additionally, protein expression of MDA5 and RIG-1 was also more strongly induced in the SOCS1 siRNA-1- and siRNA-2-treated iPS(IMR-90) cells after 6-h IFN-β treatment (Fig. 8D). Note that some STAT1 phosphorylation and ISG expression induction were observed in the negative control siRNA-treated iPS(IMR-90) cells upon IFN-β treatment (Fig. 8), which is different from our previous observation in the iPS(IMR-90) cells (Figs. 1 and 4). The reason for the base-line STAT1 phosphorylation and ISG induction in negative control siRNA-treated iPS(IMR-90) cells was likely due to a small amount of spontaneous differentiation in the population, a result of how the cells were prepared for this knockdown experiment. Taken together, these results show that the defect in STAT1 phosphorylation in human pluripotent cells and the subsequent attenuated response to type I IFNs are at least partly due to an elevated basal SOCS1 expression in these cells compared with differentiated human cells.

DISCUSSION

The basic mechanisms that regulate innate immunity are evolutionarily conserved. Cellular recognition of viral dsRNA is a crucial step of primary host defense in response to virus infection, and it leads to the production of type I IFNs. Once secreted, type I IFNs act in both autocrine and paracrine fashion to activate the intracellular JAK/STAT signaling pathway, leading to the transcription of hundreds of ISGs, thus establishing an “antiviral state” in the cell.

We previously showed that pluripotent human cells fail to respond to dsRNA due to the down-regulation of a number of genes involved in the cytoplasmic dsRNA response pathways (20). In this study, we further show that hESCs and hiPSCs have an attenuated response to type I IFNs. One of the crucial steps in IFN signaling, the activation of STAT1 phosphorylation, is...
absent in these cells upon IFN-β treatment. Although this observation can be partially explained by the moderate down-regulation of some upstream signaling molecules in the cellular type I IFN response pathway, it is significant that SOCS1, a potent inhibitor for STAT1 phosphorylation, exhibited an elevated expression level in pluripotent cells compared with their differentiated counterparts. Reduced expression of SOCS1 in hiPSCs led to a stronger response to IFN-β. Importantly, hESCs and hiPSCs share the same attenuated response to type I IFNs, suggesting an association between pluripotency and lack of IFN response in human stem cells (Fig. 9).

Human ES cells differ from mouse embryonic stem cells (mESCs) in a number of fundamental ways. For example, mESCs resemble naïve pluripotent stem cells, where activation of the LIF/STAT3 pathway is necessary and sufficient for their self-renewal and pluripotency (27–29), whereas hESCs may reflect a primed pluripotent stage, and the maintenance of their pluripotency is STAT3-independent (30, 31). In addition, previous studies indicated that mESCs are responsive to IFN-α and IFN-β (32). Here we show for the first time that human pluripotent stem cells also differ from mouse pluripotent stem cells in their response to type I IFNs.

In addition to its immunomodulatory effects, IFN signaling also contributes to several other important biological processes (for review, see Ref. 3). It has been suggested that type I IFNs can activate the mitogen-activated protein kinase p38 signaling cascade, which is not only important for IFN-dependent transcriptional activation, but also mediates stress responses in the cell (33–35). Moreover, type I IFNs also activate the phosphatidylinositol 3-kinase (PI3K) signaling cascade downstream of JAKs in an insulin receptor substrate-dependent but STAT-independent manner (36–38). Previous studies indicate that IFN-activated PI3K signaling can mediate pro-apoptotic or anti-apoptotic responses, depending on the specific cell type (39–42).
The specific action of type I IFN-mediated PI3K signaling on human pluripotent cells remains unclear and thus requires further examination. More importantly, several lines of evidence suggest that STAT1 signaling plays a pro-apoptotic role by up-regulating the expression of caspases (43–45) or by repressing pro-survival NF-κB signaling (46). Interestingly, hESCs are primed to undergo rapid apoptosis (47). It is thus possible that human pluripotent cells naturally suppress STAT1 phosphorylation to attenuate pro-apoptotic signals.

Why do human pluripotent cells have an attenuated type I IFN response? Although we do not yet know the rationale or advantage of having this lack of innate immune response, we can suggest several possibilities. First, the natural environment of human embryos or human embryonic stem cells may be well protected from foreign pathogens so these cells simply may not need to have these innate immune response mechanisms. Second, this lack of response may be crucial to maintain the integrity of the embryos as well as the organism developed from it. Thus, if the embryo is infected with a virus, it may serve the organism better for the cells to die rather than trying to fight against the infection. Third, human pluripotent cells lack the ability to respond to cytoplasmic dsRNA (20), perhaps because of a defect in the nuclear retention of dsRNAs (48), which could result in the presence of endogenous dsRNAs in the cytoplasm of these cells. As a result, cellular responses to dsRNA and the subsequent response to type I IFNs would be disadvantageous to them. Last, human embryonic stem cells are primed to undergo rapid apoptosis (47). Because there is a well established association between STAT1 activation and apoptosis, it would make sense for human pluripotent cells to down-regulate STAT1 signaling by expressing a high level of SOCS1.

Finally, several recent studies have suggested a specific way in which SOCS1 might play an important role in pluripotency. Epigenetic modifications are known to enhance reprogramming efficiency, and some of these have been shown to be associated with the activation of innate immunity (49). These chromatin modifications involve the down-regulation of histone deacetylases, leading to the activation of specific gene expression pathways. One gene activated by the inhibition of histone deacetylase is SOCS1 (49, 50). Importantly, because continued activation of innate immune signaling during reprogramming might lead to apoptosis, the activation of SOCS1 would thus promote cell survival.

Acknowledgments—We thank members of the laboratory and Dr. Renhe Xu for helpful comments on the manuscript and throughout the work.

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