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# Regulation of DC development and DC-mediated T-cell immunity via CISH

Mohammad Alam Miah[1,2] and Yong-Soo Bae[1,*]

[1]Department of Biological Sciences; Sungkyunkwan University; Suwon, Gyeonggi-do, South Korea;
[2]Department of Physiology; Bangladesh Agricultural University; Mymensingh, Bangladesh

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**Abbreviations:** CISH, cytokine inducible SH2-containing protein; CTL, cytotoxic T lymphocyte; STAT5, signal transducer and activator of transcription 5

Cytokine inducible SH2-containing protein (CISH) plays a crucial role in Type 1 dendritic cell (DC) development as well as in the DC-mediated activation of cytotoxic T lymphocytes (CTLs). CISH expression at late DC developmental stages shuts down the proliferation of DC progenitors by negatively regulating signal transducer and activator of transcription 5 (STAT5) and facilitates the differentiation of DCs into potent stimulators of CTLs.

The development of different population of dendritic cells (DCs) is controlled by cytokines, growth and differentiation factors as well as various transcription factors.[1,2] The cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF) and FLT3 ligand (FLT3L) support DC development by moldulating the expression of multiple transcription factors in DC progenitors and DCs, including PU.1, E2–2, interferon-regulatory factor (IRF)4, IRF8, SPIB, basic leucine zipper transcription factor, ATF-like (BATF) and BATF3.[3,4] In particular, GM-CSF activates the signal transducer and activator of transcription 5 (STAT5) and inhibits the FLT3L-dependent maturation of plasmacytoid dendritic cells (pDCs) by repressing IRF8.[4]

Cytokine inducible SH2-containing protein (CISH), a member of the suppressor of cytokine signaling (SOCS) protein family, has been shown to be induced in several hematopoietic cells by cytokines,[5,6] but the functional link between CISH and DC development had never been investigated. Recently, we have demonstrated that CISH is expressed and plays an important role during the GM-CSF-mediated development of DCs.[7] We found that CISH, which is not expressed in bone marrow cells, is significantly upregulated at late DC developmental stages. The levels of MHC Class I and co-stimulatory molecules were downregulated on the surface of developing DCs subjected to the CISH knockdown. The induction of CISH is involved in DC development as it inhibits the proliferation of DC precursors, allowing for their complete differentiation (**Fig. 1**). CISH is consistently upregulated by human T cells activated by interleukin (IL)-2 and is critical for both T-cell proliferation and survival upon the activation of T-cell receptor (TCR)-mediated signaling pathways.[8] Conversely, our findings suggest that CISH negatively regulates cell proliferation during DC development by blocking STAT5 activation (**Fig. 1**). CISH is well known as a STAT5 target gene, while CISH negatively regulates STAT5 activation.[9] We found that STAT5 expression and activation gradually increase during the early stages of DC development, but are suppressed by CISH at later stages, followed by the inhibition of cell proliferation (**Fig. 1**). According, the silencing of CISH led to STAT5 activation and promoted cell proliferation. These results suggest that CISH may have at least two different functions, that is, the regulation of differentiation or cell proliferation, depending on cell type and/or developmental stage. The induction of CISH plays a crucial role in the development of Type 1 DCs.

We further investigated the biological implications of CISH expression during DC development. The downregulation of MHC Class I molecules and other co-stimulatory molecules in DCs subjected to the knockdown of CISH suggested indeed that CISH might be involved in $T_H1$ immune responses. In line with this hypothesis, CISH-depleted DCs produced significantly lower amounts of the $T_H1$ cytokines IL-6, IL-12 and tumor necrosis factor α (TNFα) as compared with their control counterparts, while difference in the amounts of the $T_H2$ cytokine IL-4 less pronounced. In a T-cell proliferation assay, CISH-depleted ovalbumin (OVA)-pulsed mature DCs were significantly impaired in their ability to stimulate OVA-specific $CD8^+$ OT-I T cells as compared with control DCs, while the knockdown of CISH did not affect the ability of DCs to stimulate OVA-specific $CD4^+$ OT-II T cells. Our results suggest therefore that CISH

*Correspondence to: Yong-Soo Bae; Email: ysbae04@skku.edu
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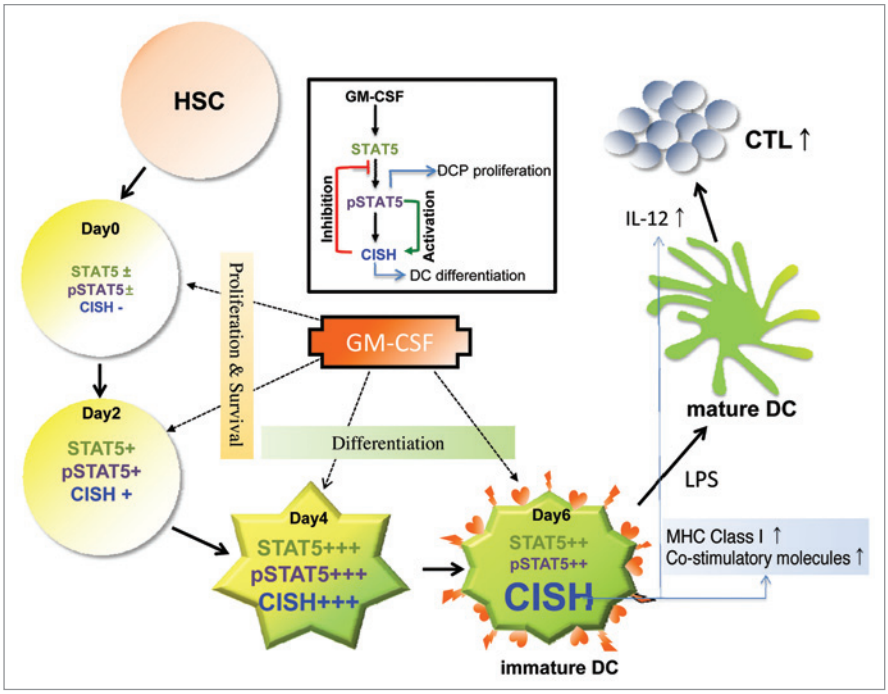


**Figure 1.** Schematic illustration of CISH expression and its function in dendritic cell development. Signaling via the granulocyte-macrophage colony-stimulating factor (GM-CSF)-signal transducer and activator of transcription (STAT) axis is well established in the development of dendritic cells (DCs) from hematopoietic stem cells (HSCs). Phosphorylated STAT5 (pSTAT5) is not only involved in the proliferation of DC precursors (DCPs) but also drives the progressive accumulation of cytokine inducible SH2-containing protein (CISH). When a threshold level is trespassed, CISH inhibits the GM-CSF-mediated activation of STAT5 in a negative feedback circuitry, resulting in the downregulation of DCP proliferation and in the differentiation of DCs into potent stimulators of cytotoxic T lymphocytes (CTLs). CISH also promotes the expression of MHC Class I and co-stimulatory molecules, as well as the production of cytokines including interleukin-12 (IL-12) when DCs are matured with lipopolysaccharide (LPS). Thus, CISH plays an important role in the late stages of DC development by acting as a molecular switch between the proliferation of DCPs and their differentiation into potent antigen-presenting DCs.

expression during the development of DCs is essential for $T_H1$ polarization.

The effectors function of DCs in DC-based vaccination models were also impaired by the depletion of CISH. In particular, the levels of interferon γ (IFNγ) in the spleen and draining lymph nodes (DLNs) of OT-1 mice vaccinated with CISH-depleted OVA-pulsed DCs were significantly lower than those of OT-1 mice vaccinated with OVA-pulsed normal DCs. OVA-specific cytotoxic T lymphocytes (CTLs) were not properly induced in mice vaccinated with CISH-depleted DCs, resulting in the impairment of DC-based immunotherapy. These data suggest that CISH is essential for the activation of $CD8^+$ T cells during DC-based immunotherapy. Overall, our findings indicate that the abundant expression of CISH during DC development is essential for the differentiation of $T_H1$ DCs from bone marrow cells, and allows for the activation of antigen-specific CTLs during DC-mediated immunotherapy.

The risk of infectious diseases appears to be increased in people carrying mutant CISH alleles.[10] This implies that CISH plays a pivotal role in the immune response against foreign invaders, but the underlying immunological mechanisms remain unclear. It has been proposed that CISH mutations may be accompanied by enhanced STAT5 activation, leading to an increase in regulatory T cells (Tregs), and hence to immunosuppressive effects that may enhance the susceptibility to infectious agents. Our findings suggest that the relatively high susceptibility to infectious diseases of patients bearing *CISH* mutations is at least in part due to an impaired induction of CTL responses by DCs. *CISH*$^{-/-}$ mice if available will surely provide a better understanding of the role of CISH in immune responses in vivo.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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