Increased nuclear suppressor of cytokine signaling 1 in asthmatic bronchial epithelium suppresses rhinovirus induction of innate interferons

Vera Gielen, MSc,[a,b,c] Annemarie Sykes, MD,[a,b,c,d] Jie Zhu, PhD,[a,b,c] Brian Chan, BSc,[a] Jonathan Macintyre, MD,[a,b,c,d] Nicolas Regamey, MD,[e] Elisabeth Kieninger, MD,[e] Atul Gupta, MD, PhD,[b,f] Amelia Shoemark, PhD,[b,f] Cara Bossley, MD,[b,f] Jane Davies, MD, PhD,[b,f] Sejal Saglani, MD, PhD,[b,f] Patrick Walker, PhD,[g] Sandra E. Nicholson, PhD,[h,i] Alexander H. Dalpke, MD, PhD,[g] Onn-Min Kon, MD, PhD,[d] Andrew Bush, MD, PhD,[b,f] Sebastian L. Johnston, MD, PhD,[a,b,c,d]* and Michael R. Edwards, PhD[a,b,c]* *London, United Kingdom, Bern, Switzerland, Heidelberg, Germany, and Parkville, Australia*

Background: Rhinovirus infections are the dominant cause of asthma exacerbations, and deficient virus induction of IFN-α/β/λ in asthmatic patients is important in asthma exacerbation pathogenesis. Mechanisms causing this interferon deficiency in asthmatic patients are unknown.
Objective: We sought to investigate the expression of suppressor of cytokine signaling (SOCS) 1 in tissues from asthmatic patients and its possible role in impaired virus-induced interferon induction in these patients.
Methods: We assessed *SOCS1* mRNA and protein levels *in vitro*, bronchial biopsy specimens, and mice. The role of SOCS1 was inferred by proof-of-concept studies using overexpression with reporter genes and *SOCS1*-deficient mice. A nuclear role of SOCS1 was shown by using bronchial biopsy staining, overexpression of mutant SOCS1 constructs, and confocal microscopy. SOCS1 levels were also correlated with asthma-related clinical outcomes.
Results: We report induction of SOCS1 in bronchial epithelial cells (BECs) by asthma exacerbation–related cytokines and by rhinovirus infection *in vitro*. We found that SOCS1 was increased *in vivo* in bronchial epithelium and related to asthma severity. *SOCS1* expression was also increased in primary BECs from asthmatic patients *ex vivo* and was related to interferon deficiency and increased viral replication. In primary human epithelium, mouse lung macrophages, and *SOCS1*-deficient mice, SOCS1 suppressed rhinovirus induction of interferons. Suppression of virus-induced interferon levels was dependent on SOCS1 nuclear translocation but independent of proteasomal degradation of transcription factors. Nuclear SOCS1 levels were also increased in BECs from asthmatic patients.
Conclusion: We describe a novel mechanism explaining interferon deficiency in asthmatic patients through a novel nuclear function of SOCS1 and identify SOCS1 as an important therapeutic target for asthma exacerbations. (J Allergy Clin Immunol 2015;136:177-88.)

Key words: *Rhinovirus, asthma, asthma exacerbation, atopy, interferon, innate immunity, cytokine, T_H2 inflammation, suppressor of cytokine signaling*

Asthma exacerbations are the major cause of morbidity, mortality, and health care costs in asthmatic patients and cause a decrease in lung function.[1] Respiratory tract virus infections, of

From [a]the Airway Disease Infection Section and [f]Respiratory Pediatrics, National Heart and Lung Institute, and [c]the Centre for Respiratory Infection, Imperial College London; [b]MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, London; [d]Imperial College Healthcare National Health Service Trust, London; [e]Pediatric Medicine, University of Bern; [g]the Department of Infectious Diseases, Medical Microbiology and Hygiene, University of Heidelberg; [h]the Walter & Eliza Hall Institute, Parkville; and [i]the Department of Medical Biology of the University of Melbourne, Parkville.

*These authors contributed equally to this work.

V.G. was funded by a studentship from the National Heart Lung Institute Foundation, Imperial College London. M.R.E. was supported by a Fellowship and S.L.J. was supported by a Chair from Asthma UK (RF07_04, CH11SJ respectively). A.S. was funded by a MRC Clinical Research Fellowship. This work was supported in part by grants from the British Lung Foundation (P06/13), MRC project grant G0601236, MRC Centre grant G1000758, ERC FP7 Advanced grant 233015 (to SLJ), the National Institute of Health Research Biomedical Research Centre funding scheme, National Institute of Health Research BRC Project grant P26095, Predicta FP7 Collaborative Project grant 260895, and the Wellcome Trust–sponsored Centre for Respiratory Infection (CRI). A.B. was supported by the National Institute of Health Research Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London. P.W. was supported by the German Research Foundation grants Da592/4 and SFB938. S.E.N. was supported by National Health and Medical Research Council Program grants 461219 and 1016647.

Disclosure of potential conflict of interest: V. Gielen has received research support from the National Heart and Lung Institute. A. Sykes has received research support from the Academy of Medical Sciences–Clinical Lecturer starter grant and MRC Clinical Training Fellowship. J. Davies has consultant arrangements with Vertex Pharmaceuticals, Novartis, PTC, and Bayer; has provided an EMA review of Vertex investigation plans; has received payment for lectures from Vertex, Forest, Novartis, and Bayer; and has received payment for development of educational presentations from Vertex and the European Cystic Fibrosis Society. S. E. Nicholson has received research support from the National Health and Medical Research Council, Australia. A. H. Dalpke has received research support from the German Research Foundation (DFG, Da592/4, SFB938) and Gilead. O.-M. Kon has received research support from a Boehringer Ingelheim ERS 2014 travel grant. S. L. Johnston has received research support and consultant fees from Centocor, Sanofi Pasteur, GlaxoSmithKline, Chiesi, Boehringer Ingelheim, and Novartis; has received research support and consultant fees from and is a shareholder in Synairgen; has received consultant fees from Grunenthal and Bioforce; and has UK, international, European, Japanese, Hong Kong, and US patents. M. R. Edwards has received research support from the Asthma UK Fellowship (RF07_04), the Medical Research Council (G0601236), and the British Lung Foundation (P06/13). B. Chan did not return a conflict of interest disclosure statement. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 27, 2014; revised October 27, 2014; accepted for publication November 12, 2014.

Available online January 25, 2015.

Corresponding author: Michael R. Edwards, PhD, Airway Disease Infection Section, National Heart and Lung Institute, St Mary's Campus, Imperial College London, London W2 1PG, United Kingdom. E-mail: michael.edwards@imperial.ac.uk.

0091-6749

http://dx.doi.org/10.1016/j.jaci.2014.11.039

Abbreviations used
AA: Atopic asthma
BAL: Bronchoalveolar lavage
BEC: Bronchial epithelial cell
CISH: Cytokine-inducible SH2-containing protein
GFP: Green fluorescent protein
ISG: Interferon-stimulated gene
ISRE: Interferon-stimulated response element
KC: Keratinocyte-derived chemokine
LIX: LPS-induced CXC chemokine
NANA: Nonatopic nonasthmatic
NF-κB: Nuclear factor κB
NLS: Nuclear localization sequence
polyI:C: Polyinosinic-polycytidylic acid
SOCS: Suppressor of cytokine signaling
SOCS1wt: Full-length wild-type human SOCS1
STAT: Signal transducer and activator of transcription
STRA: Severe therapy-resistant atopic asthma

which human rhinoviruses are by far the most common,[2,3] cause the great majority of asthma exacerbations. The mechanisms involved in asthma exacerbations are poorly understood, but increased susceptibility to rhinovirus infections is strongly implicated.[4,5]

We originally reported impaired induction of the innate antiviral IFN-β[6] and IFN-λ[7] by rhinovirus infection in lung cells from asthmatic patients and implicated deficiency of IFN-λ in asthma exacerbation severity in human subjects *in vivo*.[7] Recent studies have confirmed deficient respiratory tract virus induction of IFN-α, IFN-β, and/or IFN-λ in bronchial epithelial cells (BECs), bronchoalveolar lavage (BAL) macrophages, peripheral blood dendritic cells, and PBMCs from asthmatic patients.[8-14] Although impaired interferon induction might be associated with asthma control,[15] the mechanism or mechanisms responsible for impaired interferon induction are currently unknown. Two recent studies reported that exogenous TGF-β enhanced rhinovirus replication in fibroblasts and BECs and that this was accompanied by reduced interferon levels.[16,17] The latter study also reported that anti–TGF-β treatment of BECs from asthmatic patients was accompanied by reduced suppressor of cytokine signaling *(SOCS) 1* and *SOCS3* gene expression,[17] possibly associating these SOCS proteins with interferon deficiency, but no investigations of SOCS function were performed.

There are 7 SOCS family members in human subjects and mice: SOCS1 through SOCS6 and cytokine-inducible SH2-containing protein (CISH). The family is characterized by a central SH2 domain and a C-terminal SOCS box motif that couples SOCS proteins to a Cullin-RING E3 ubiquitin ligase complex. Therefore SOCS proteins can act as adaptors to target bound proteins for ubiquitination and proteasomal degradation and thus function as negative regulators of cytokine signaling. SOCS1 through SOCS3 have been studied in detail, including development of knockout mice.[18-20] *SOCS1* deletion causes fatal inflammation, which can be rescued by deletion of *IFNG*.[18] In mice SOCS1 and SOCS2 negatively regulate T_H2 immunity[19,21-23]; however, a human polymorphism enhancing SOCS1 expression is associated with asthma.[24] T-cell SOCS3 mRNA levels are increased in asthmatic patients and correlate with IgE levels,[20] but a functional role for SOCS3 in human asthma is unknown, and thus the role of SOCS proteins in asthma is unclear.

In the context of viral infections, SOCS proteins suppress cytokine receptor signaling through inhibition of Janus-activated kinase and signal transducer and activator of transcription (STAT) signaling,[25-27] and preliminary data suggest that SOCS1 and SOCS3 might suppress influenza-induced IFN-β promoter activation.[28] However, there are no data on the possible role of SOCS proteins in suppressing viral induction of interferons in patients with asthma and during asthma exacerbations.

We hypothesized that SOCS1/3 would be induced by proinflammatory cytokines and rhinovirus infection in BECs *in vitro*. Thus we investigated SOCS expression in human primary BECs from asthmatic patients *ex vivo* and their possible role in interferon deficiency and increased viral replication in these cells. We also investigated whether SOCS1/3 proteins could directly suppress viral induction of innate interferons in airway cells *in vitro* and *in vivo*. We found that SOCS1, but not SOCS3, levels were increased in cells from asthmatic patients and also found that nuclear localization of SOCS1 was required for suppression of virus-induced interferons. This suppression was independent of the only known nuclear function of SOCS1, which is induction of proteasomal degradation of signaling proteins. Thus we describe a novel mechanism explaining interferon deficiency in asthmatic patients, a new nuclear function of SOCS1, and identify SOCS1 as an important therapeutic target for asthma exacerbations.

METHODS

For detailed methods, including patient data, animal models, reagents, experimental protocols, and statistical analysis, please see the Methods section and Tables E1-E3 in this article's Online Repository at www.jacionline.org.

RESULTS

SOCS1 is induced in primary BECs by proinflammatory cytokines and rhinovirus

SOCS3 mRNA expression is increased in T cells in asthmatic patients,[20] but upregulation of SOCS1 by IL-13 in airway smooth muscle cells from asthmatic patients is impaired.[22] Thus whether SOCS proteins are upregulated in asthmatic patients is uncertain, and whether SOCS proteins are upregulated in cells that are infected by respiratory tract viruses is unknown. Therefore we first investigated the effects of the T_H2 cytokines IL-4 and IL-13 on *SOCS1* through *SOCS6* and *CISH* mRNA and protein expression in BECs because these cytokines are strongly implicated in asthma pathogenesis.[29,30] IL-4 and IL-13 both induced *SOCS1* mRNA and protein expression (Fig 1, *A*). Densitometric analysis for the Western blots in Fig 1 are shown in Fig E1 in this article's Online Repository at www.jacionline.org. No other SOCS proteins/mRNAs were induced by IL-4 or IL-13, with the exception of CISH, which was significantly induced by both.

We next investigated the ability of the proinflammatory cytokines TNF-α and IL-1β, rhinovirus infection, and polyinosinic-polycytidylic acid (polyI:C; as a mimic of other viral infections) to induce SOCS expression in BECs. We found that TNF-α and IL-1β both induced SOCS1 (Fig 1, *B*) but not any other SOCS family member, whereas both SOCS1 (Fig 1, *C*) and SOCS3 (see Fig E2 in this article's Online Repository at www.jacionline.org) were induced by RV1B (representative of minor group rhinoviruses), RV16 (major group), and polyI:C. RV1B