SOCS3 Deletion in B Cells Alters Cytokine Responses and Germinal Center Output

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B cell behavior is fine-tuned by internal regulatory mechanisms and external cues such as cytokines and chemokines. Suppressor of cytokine signaling 3 (SOCS3) is a key regulator of STAT3-dependent cytokine responses in many cell types and has been reported to inhibit CXCL12-induced retention of immature B cells in the bone marrow. Using mice with SOCS3 exclusively deleted in the B cell lineage (Socs3Δ/Δmb1cre†), we analyzed the role of SOCS3 in the response of these cells to CXCL12 and the STAT3-inducing cytokines IL-6 and IL-21. Our findings refute a B cell-intrinsic role for SOCS3 in B cell development, because SOCS3 deletion in the B lineage did not affect B cell populations in naive mice. SOCS3 was strongly induced in B cells stimulated with IL-21 and in plasma cells exposed to IL-6. Its deletion permitted excessive and prolonged STAT3 signaling following IL-6 stimulation of plasma cells and, in a T cell-dependent immunization model, reduced the number of germinal center B cells formed and altered the production of Ag-specific IgM and IgE. These data demonstrate a novel regulatory signal transduction circuit in plasma cells, providing, to our knowledge, the first evidence of how these long-lived, sessile cells respond to the external signals that mediate their longevity. *The Journal of Immunology, 2011, 187: 000–000.

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Abbreviations used in this article: BM, bone marrow; ES, embryonic stem; GC, germinal center; HPRT1, hypoxanthine guanine phosphoribosyl transferase 1; KLH, keyhole limpet hemocyanin; MMTV, mouse mammary tumor virus; NP, (4-hydroxy-3-nitrophenyl)acetetyl; QPCR, quantitative PCR; SHM, somatic hypermutation; SOCS3, suppressor of cytokine signaling 3.

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binase from the mbl1 locus (21). The mbl1 gene encodes CD79α, an intracellular component of the BCR, which is expressed exclusively in B cells beginning at the pro-B cell stage (21). Our data refute the finding that SOCS3 expression in immature B cells is a prerequisite for their timely exit from the BM (9) and shows that SOCS3 regulates both the maintenance of the GC and IgE affinity maturation during a T cell-dependent immune response.

Materials and Methods

Mice

Mice with floxed (20) or null (22) alleles of Socs3 were crossed with mice expressing cre recombining under the mbl1 promoter (21) or the MMTV promoter (10). All mice were on a C57BL/6 background and were maintained in a conventional animal facility. All procedures were performed in compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Melbourne Health Animal Ethics Committee.

Abs and flow cytometry for identification of B cell subsets

Single-cell suspensions from BM or spleen were treated to lyse RBCs and stained with Ab conjugates for flow cytometry, including anti-CD19 (clone ID3), anti-CD21 (clone 7G6), anti-CD23 (clone B3B4), anti-B220 (clone RA3-6B2), anti-CD5 (clone 53-7,3), and anti–Synd-1 (clone 281; CD138), all purified and conjugated in our laboratory or purchased from BD Pharmingen. Avidin–Cy5 was obtained from Southern Biotechnology Associates. B cell subsets were identified based on surface marker expression. B cells were identified as B220+IgM+IgD+ (a population that may also contain some macrophage precursors, dendritic cells, and NK cells), immature B cells as B220+IgM-IgD+, and recirculating B cells as B220+IgM+IgD+. In spleen, transitional 1 (T1) B cells were identified as B220+IgM+IgD+CD21-CD23+, transitional 2 (T2) B cells as B220+IgM+IgD-CD21+CD23-, marginal zone B cells as B220+IgM+IgD-CD21-CD23+, and follicular B cells as B220+IgM+IgD+CD21+CD23+. Plasma cells were identified as B220+IgM+IgD-CD38-. Flow cytometry was performed on an LSR II or FACS Calibur (BD Biosciences) cytometer, and data on at least 10⁶ viable cells, determined by propidium iodide exclusion, were collected. Live cells were sorted on the basis of propidium iodide exclusion on a FACS Diva (BD Instruments) or MoFlo (DakoCytomation) cytometer.

Quantification of gene expression

To measure cytokine induction of Socs3, IL-21R, IL-6Rα, or Bcl-6 mRNA expression, cells were FACSorted as above and then stimulated with either 10 ng/ml recombinant mouse IL-21 (a gift from Zymogenetics) or 10 ng/ml IL-6 [supernatant from transfected hybridoma cell line, optimal concentration determined by cell culture (23)]. Total RNA was isolated from sorted B cells or plasma cells using an RNeasy Mini Kit (Qiagen) and quantified by spectrophotometer at 260 nm absorbance. With 100–600 ng total RNA with equal amounts for comparable samples and the calibrator sample, cDNA was synthesized using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The calibrator sample for expression of Socs3 and IL-6Rα was total RNA isolated from murine ES cells stimulated in vitro with LIF for 30 min. The calibrator sample for expression of Bcl-6 was total RNA isolated from murine ES cells stimulated in vitro with LIF for 30 min. The calibrator sample for expression of Bcl-6 was total RNA isolated from wild-type skeletal muscle. The calibrator sample for expression of IL-21R was FACSorted wild-type unstimulated B cells (B220+Syd-1-). The negative control was the calibrator sample reaction without reverse transcriptase.

Real-time quantitative PCR (QPCR) was performed in triplicate or quadruplicate using TaqMan Gene Expression Assays (Applied Biosystems) for Socs3 (Assay ID: Mm01249143_m1), IL-21R (Assay ID: Mm00600319_m1), IL-6Rα (Assay ID: Mm00439653_m1), Bcl-6 (Assay ID: Mm00477633_m1), or the endogenous control hypoxanthine guanine phosphoribosyl transferase 1 (HPRT1) (Assay ID: Mm00138743_m1), using an Applied Biosystems 7900HT sequence detection system. Data were analyzed using SDS 2.2 software (Applied Biosystems) and the Relative Quantification (ΔΔCt) method. Data shown are as mean relative expression of Socs3, normalized for HPRT1 expression, compared with the calibrator sample.

Detection of intracellular phosphorylated STAT3

Detection of phosphorylated STAT3 by flow cytometry was achieved using Abs (anti-IgM, anti-IgG1, or anti-IgE) directly coupled to HRP (Southern Biochemicals, Cleveland, U.K.), precipitated in alum. The Ag was coupled to BSA at either a high (NP13-BSA) or low (NP 2-BSA) haptenation rate was used as a plate coat. B220+ cells were then examined for their expression of surface IgG1 and their ability to bind NP directly conjugated to PE. Cells with both these characteristics were then further subdivided, based on CD38 expression into GC (CD38+) or memory (CD38-) subsets.

ELISA and ELISPOT assay

For ELISA, serum was isolated from non–heparin-treated blood by allowing blood to clot for 4 h at 4°C, then centrifuging at 13,500 rpm for 7 min. The supernatant serum was removed and stored at −20°C. Serum Ig concentration was measured using ELISA, and Ig-secreting cells were counted by ELISPOT as described previously (25).

For capture of Ag-specific IgG1, NP coupled to BSA at either a high (NP13-BSA) or low (NP-BSA) haptenation rate was used as a plate coat for ELISAs or ELISPOTs, as described previously (24). Detection of anti-NP Abs of different isotypes was achieved using isotype-specific secondary Abs (anti-IgM, anti-IgG1, or anti-IgE) directly coupled to HRP (Southern Biotechnology Associates).

Cell culture

Spleen B cells (CD19+) were cultured in triplicate at 5 × 10⁴ or 3 × 10⁴ cells in 200 μl RPMI 1640 medium plus 5% FCS plus 100 μM 2-ME with an optimal concentration of baculovirus-derived CD40L (determined by cell culture), IL-4 (100 U; PeproTech), and IL-21 (10 ng/ml) for 4 d at 37°C with 10% CO₂. Following this culture period, a known number of CaliBRITE beads (BD Biosciences) was added to each well, and the live cell number was determined on the basis of propidium iodide exclusion by flow cytometry.

Cell cycle analysis

Between 20,000 and 50,000 GC B cells were sorted per mouse, washed, and resuspended in 100 μl propidium iodide buffer (0.1% sodium acetate, 0.2% Triton X-100, 10 μg/ml RNase A, and 50 μg/ml propidium iodide). Cells were vortexed twice for 10 s then incubated at room temperature for 20 min in the dark before analysis on a Canto II cytometer (BD Instruments). At least 10,000 events were recorded, and data were analyzed using FlowJo version 8.8.2 cell cycle analysis software (Tree Star).

Statistical analyses

Unpaired, two-tailed t tests were performed using Microsoft Excel software. Differences were deemed significant where p < 0.05.

Results

SOCS3 expression is developmentally regulated in B cells and plasma cells and is further induced by IL-6 and IL-21.

Given that SOCS3 expression has been reported to increase as a B cell progresses through developmental stages in the BM (9), we
assessed the consequences of B cell-specific SOCS3 deletion on B cell development and differentiation. B cells of different subsets were sorted from BM and spleen, and SOCS3 mRNA expression was measured by QPCR (Fig. 1A). Pre-/pro-B cells and immature B cells in the BM contained a low level of SOCS3 mRNA, which was increased 3-fold (compared with pre-/pro-B cells) in recirculating mature B cells in the BM, 6-fold in follicular B cells and 4-fold in plasma cells in the spleen (Fig. 1A).

SOCS3 mRNA expression was dramatically induced by IL-21 in wild-type splenic B cells, increasing 25-fold after 30 min relative to B cells at rest (Fig. 1B). This effect was not observed in SoCS3^{Δ/Δ} mblcre splenic B cells, demonstrating efficient mblcre–mediated deletion of the loxP-flanked Socs3 locus in these cells (Fig. 1B). The lack of SOCS3 mRNA induction with IL-21 stimulation was not due to downregulation of IL-21R, because expression of IL-21R and IL-6R α was not due to downregulation of IL-21R, because expression of the IL-21 target gene Bcl-6 was not affected by SOCS3 deficiency in plasma cells (Fig. 2A). Consistent with this finding, expression of the IL-21 target gene Bcl-6 was not affected by SOCS3 deletion (Fig. 2E). This indicates the involvement of altemative regulatory mechanisms in limiting IL-21–induced STAT3 activation and downstream target gene effects in B cells.

In plasma cells, interaction with BM stromal cells induces expression of IL-6, which may act to enhance plasma cell survival (26). As IL-6 signaling through STAT3 is regulated by SOCS3 in macrophages (2, 3, 5), we examined whether SOCS3 regulation of STAT3 phosphorylation occurred in plasma cells. SOCS3 deficiency in plasma cells resulted in elevated and sustained levels of phosphorylated STAT3 following their stimulation with IL-6 (Fig. 2D). To our knowledge, these data are the first to demonstrate that in plasma cells, as in macrophages, IL-6 induces phosphorylation of STAT3, causing expression of SOCS3, and that SOCS3 then attenuates signaling through this pathway by truncating STAT3 activity.

**Deletion of SOCS3 does not impair B cell development or response to CXCL12**

With MMTV-cre–mediated deletion, SOCS3 deficiency has been reported to cause a 2-fold accumulation of immature B cells in the BM (9), although this finding cannot be attributed to the loss of SOCS3 only in B cells. To isolate B cell-intrinsic effects of SOCS3 deficiency from those due to perturbations in the BM stromal environment, to which developing B cells are highly sensitive, B cell development was examined in SoCs3^{Δ/Δ} mblcre (SOCs3^{+/+}) and SoCs3^{Δ/Δ} mblcre (SOCs3^{Δ/Δ}) mice. In these mice, no differences were observed compared with wild-type in the number or proportion of immature B cells or any other subset in the BM, and there were no differences detected in the sizes of splenic B cell populations (Fig. 3A). Moreover, in our hands, there were no changes detected in the number or proportion of immature B cells in the BM of SoCs3^{Δ/Δ} MMTVcre (SOCs3^{Δ/Δ}) mice (Fig. 3B), in contrast to what has been reported previously (9).

**FIGURE 1.** SOCS3 is expressed in B cells and plasma cells and is further induced by cytokines. Data in each panel are expressed as mean relative SOCS3, IL-21R, or IL-6Rα expression, normalized for HPRT1 expression, compared with the calibrator sample, as described in Materials and Methods.

A and B. Bars represent mean ± SEM from cells from three to five mice. A, SOCS3 mRNA expression in FACS-sorted wild-type B cell populations: pre-/pro-B (BM, B220^{IgM-1gD-}), immature (BM, B220^{IgM-1gD-}), recirculating (BM, B220^{IgM-1gD-}), follicular (spleen, B220^{IgM-1gD-}CD21^{+}CD23^{+}), and plasma cells (spleen, B220^{IgM-1gD-}Synd-1^{+}). B, FACS-sorted naive B cells (B220^{IgM-1gD-}Synd-1^{+}) from SoCs3^{+/+} mblcre (SOCs3^{+/+}) or SoCs3^{Δ/Δ} mblcre (SOCs3^{Δ/Δ}) mice were stimulated with 10 ng/ml IL-21 for the indicated times in minutes, and SOCS3 mRNA expression was measured by QPCR (*p < 0.01 versus unstimulated SOCS3^{+/+}). C, FACS-sorted naive B cells (B220^{IgM-1gD-}Synd-1^{+}) from SoCs3^{Δ/Δ} mblcre (SOCs3^{Δ/Δ}; n = 3) were stimulated with 10 ng/ml IL-21 for the indicated times in minutes. IL-21R mRNA expression was measured by QPCR. D, FACS-sorted naive B cells (B220^{IgM-1gD-}Synd-1^{+}) from SoCs3^{Δ/Δ} mblcre (SOCs3^{Δ/Δ}; n = 3) were stimulated with 10 ng/ml IL-21 for the indicated times in minutes, and IL-6Rα mRNA expression was measured by QPCR.
Thus, our data do not support a role for B cell-expressed SOCS3 in regulating immature B cell development.

As well as its roles in polarizing the GC reaction and in directing plasma cells to the BM, the chemokine CXCL12 has been shown to retain developing B cells in the BM until they reach the immature stage, a process reported to be subject to SOCS3 regulation (9). To examine whether SOCS3 deletion only in B cells alters their responsiveness to CXCL12, BM B cell populations were sorted from Socs3fl/fl; mb1cre (SOCS3Δ/Δ) and Socs3fl/fl (SOCS3+/+) mice and a Transwell assay performed to assess specific migration toward CXCL12 (Fig. 3C). The rate of migration of immature B cells toward CXCL12 was lower than that of pre-/pro-B cells or recirculating mature B cells, indicating that a reduced response to CXCL12 may indeed be associated with BM egress of immature B cells (Fig. 3C). Loss of SOCS3, however, did not change the extent of migration of any B cell subset toward CXCL12, arguing against a role for B cell-expressed SOCS3 in regulating this response. Furthermore, the frequency of plasma cells in the BM, which are dependent on CXCL12 for their initial BM homing and subsequent persistence, was unchanged in the absence of SOCS3 (Fig. 3A). It appears, therefore, that SOCS3 produced by the B lineage is not a critical regulator of B cell responsiveness to CXCL12 migration or survival signals.

**SOCS3 deficiency does not affect steady-state plasma cell formation or survival**

To assess the cellular effects of IL-6 hyperresponsiveness in SOCS3 deficient plasma cells, we measured the number of plasma cells in the BM and spleen of Socs3fl/fl; mb1cre (SOCS3Δ/Δ) and Socs3fl/fl (SOCS3+/+) mice as well as their isotype profile and Ig secretion. Despite our finding that SOCS3 regulates IL-6-induced STAT3 activation (Fig. 2D), deletion of SOCS3 did not alter the number or proportion of plasma cells in the BM and spleen of naïve animals (Fig. 3A). The absence of SOCS3 and resultant increase in STAT3 signaling did not change the amount of IgG in the serum (Fig. 4).

Humans with STAT3 deficiency develop hyper-IgE syndrome (27) and deficiency of IL-21, which signals through STAT3, is also associated with hypersecretion of IgE (16, 28, 29). These observations suggest that alterations to STAT3 signaling in the absence of SOCS3 might perturb serum IgE levels. However, no differences were detected in IgE serum levels when SOCS3 was deleted...
in the B lineage, and there were no other significant differences in the serum levels of other Ab isotypes (Fig. 4).

**SOC3 in B cells sustains the GC reaction in response to T cell-dependent immunization**

There is significant interest in the roles of IL-21 and STAT3 signaling in maintaining the GC reaction and promoting appropriately timed plasma cell differentiation (7, 16, 17). As our data showed that SOCS3 is induced upon IL-21 stimulation of B cells, we examined the effect of SOCS3 deficiency on the humoral response to immunization with NP-KLH. GC B cells were enumerated by flow cytometry by identifying (IgM, IgD, Synd-1, and Gr-1) negative, B220 + cells (B220 +IgM+IgD+). Frequencies of B cell populations in the BM and spleen were determined by FACS in wild-type mice (Socs3Δfl/+; SOCS3+/+, mice lacking one allele of Socs3 in B cells (Socs3Δfl/+; SOCS3+/−); Socs3Δfl/fl; SOCS3Δ/Δ) or mice heterozygous for Socs3 in all other cell types but lacking both alleles of Socs3 in B cells (Socs3Δfl/fl; SOCS3Δ/Δ); BM B cell populations were determined by FACS in wild-type mice (Socs3Δfl/+; SOCS3+/+, mice lacking one allele of Socs3 in B cells (Socs3Δfl/+; SOCS3+/−); Socs3Δfl/fl; SOCS3Δ/Δ) or mice in which Socs3 was deleted using MMTV-cre (Socs3Δfl/MMTVcre; SOCS3Δ/Δ). B cell populations were sorted by FACS from the BM of Socs3Δfl/fl; MMTVcre−/+ (Socs3Δ/Δ) or Socs3Δfl/fl; MMTVcre−/− (Socs3ΔΔ) mice and subjected to Transwell migration assay where specific migration toward CXCL12 was determined over a 4-h period. There were no statistically significant differences in migration toward CXCL12 in SOCS3Δ/Δ compared with SOCS3Δ/Δ for any of the B cell subsets tested. In each panel, bars represent mean ± SEM for cells from three to five mice.

### FIGURE 4

Absence of SOCS3 does not alter steady-state Ab production. Serum was extracted from nonimmunized Socs3Δfl/fl; mb1cre−/+ (Socs3Δ/Δ), Socs3Δfl/fl; mb1cre−/− (Socs3ΔΔ), or Socs3Δfl/fl; mb1cre−/+ (Socs3ΔΔ) mice, and Ab levels were determined by ELISA. Bars represent mean plus SEM of serum from six to nine mice.

### FIGURE 3

SOC3 deficiency does not alter B cell development or migration. B cells in the BM were identified as pre/pro-B (B220+IgM−IgD−), immature (B220+IgM+IgD−), recirculating (B220+IgM+IgD+), or plasma cells (B220+IgM+IgD+) by FACS in wild-type mice (Socs3Δfl/+; SOCS3+/+, mice lacking one allele of Socs3 in B cells (Socs3Δfl/+; SOCS3+/−); Socs3Δfl/fl; SOCS3Δ/Δ), and B cells in the spleen were identified as T1 (B220+IgM+IgD−CD21−CD23−), T2 (B220+IgM+IgD+CD21hiCD23+), marginal zone (B220+IgM+IgD+CD21hiCD23+), follicular (B220+IgM+IgD+CD21hiCD23+), or plasma cells (B220+IgM+IgD+). Frequencies of B cell populations in the BM and spleen were determined by FACS in wild-type mice (Socs3Δfl/+; SOCS3+/+, mice lacking one allele of Socs3 in B cells (Socs3Δfl/+; SOCS3+/−); Socs3Δfl/fl; SOCS3Δ/Δ) or mice heterozygous for Socs3 in all other cell types but lacking both alleles of Socs3 in B cells (Socs3Δfl/fl; SOCS3Δ/Δ); BM B cell populations were determined by FACS in wild-type mice (Socs3Δfl/+; SOCS3+/+, mice lacking one allele of Socs3 in B cells (Socs3Δfl/+; SOCS3+/−); Socs3Δfl/fl; SOCS3Δ/Δ) or mice in which Socs3 was deleted using MMTV-cre (Socs3Δfl/MMTVcre; SOCS3Δ/Δ). B cell populations were sorted by FACS from the BM of Socs3Δfl/fl; MMTVcre−/+ (Socs3Δ/Δ) or Socs3Δfl/fl; MMTVcre−/− (Socs3ΔΔ) mice and subjected to Transwell migration assay where specific migration toward CXCL12 was determined over a 4-h period. There were no statistically significant differences in migration toward CXCL12 in SOCS3Δ/Δ compared with SOCS3Δ/Δ for any of the B cell subsets tested. In each panel, bars represent mean ± SEM for cells from three to five mice.
significant effects on the IgG1 response. Immunization with a T cell-dependent Ag, but its deletion has no modulates the production of Ag-specific IgM and IgE in response to a T cell-dependent Ab response. We have also shown that SOCS3 in maintaining GC B cell numbers during the late phase of reaching a plateau in wild-type mice (Fig. 7C) which continued to increase in the SOCS3-deleted animals after the response progressed (Fig. 7C). This was particularly apparent when the analysis was restricted to anti-NP IgE of high affinity, which continued to increase in the SOCS3-deleted animals after reaching a plateau in wild-type mice (Fig. 7C). Taken together, these data reveal a B cell-intrinsic role for SOCS3 in maintaining GC B cell numbers during the late phase of a T cell-dependent Ab response. We have also shown that SOCS3 modulates the production of Ag-specific IgM and IgE in response to immunization with a T cell-dependent Ag, but its deletion has no significant effects on the IgG1 response.

Discussion
Fine regulation of the formation and behavior of B cells and their responsiveness to various immune environments are achieved through the activity of external factors including cytokines and chemokines. Several of these factors act via a STAT3-dependent signal transduction pathway. Because SOCS3 is a key feedback inhibitor of STAT3 signaling, particularly in response to IL-6 stimulation of macrophages (2, 3, 5), we sought to determine whether this inhibitory system extended to the B cell lineage and whether deletion of SOCS3 would impact facets of B cell behavior that are influenced by STAT3-inducing stimuli.

SOCS3 expression was low in pre-/pro-B cells and immature B cells and increased as the cells matured, supporting previously published data (9). Using MMTV-cre-mediated SOCS3 deletion, Le et al. (9) found that SOCS3 inhibited adhesion responses of developing B cells in the BM and allowed their timely egress to the periphery. SOCS3 deletion was reported to cause a 2-fold increase in the number of immature B cells retained in the BM (9). The use of MMTV-driven cre recombinase expression, however,
will result in SOCS3 deletion in a number of hemopoietic cell types, including B and T cells, megakaryocytes, and erythroid cells (10). Although this level of deletion avoids the embryonic lethality that occurs in mice lacking SOCS3 in all tissues (22, 32), Socs3^{fl/fl} MMTVcre^+ mice show evidence of disrupted immune function as a result of altered chemokine secretion and neutrophil responses (33, 34). We have shown that when SOCS3 is deleted exclusively in B cells using mb1-cre, there is no accumulation of immature B cells in the BM. In addition, in our colonies of Socs3^{fl/fl} MMTVcre^+ mice, numbers of immature B cell populations were not different from wild-type (Fig. 3B). Therefore, evidence presented in this article is not consistent with the finding that SOCS3 deficiency in immature B cells prevents them from leaving the BM (9) and points to a role for SOCS3 in other cell types as the explanation for this phenomenon.

In mature B cells, the level of SOCS3 mRNA expression was around half that produced by ES cells stimulated with LIF. Upon exposure to IL-6, SOCS3 expression by B cells increased 5-fold over 60 min, whereas IL-21 raised SOCS3 transcript levels 25-fold within 30 min. A role for IL-21 has recently been described in maintaining B cells as they progress through the GC reaction (16, 17). GC B cells are subject to major assault on the integrity of genes at the Ig loci during the process of SHM and class-switch recombination. While this process occurs and the cell cycle is halted, IL-21 signaling prevents apoptosis of GC B cells (16, 17). This is thought to occur mainly via the induction of Bcl-6 by IL-21–stimulated STAT3 activation (18, 35, 36), although STAT3 deficiency in B cells does not fully recapitulate the phenotype of mice with impaired IL-21 signaling (7, 16, 17).

Our data show that SOCS3 deficiency does not alter STAT3 phosphorylation or Bcl-6 expression following IL-21 stimulation of mature B cells, indicating that alternative mechanisms can modulate signaling in this pathway when SOCS3 is absent. This is not the case for the IL-6 pathway in plasma cells, where we found that SOCS3 was required for the regulation of STAT3 phosphorylation—a phenomenon that is also observed in macrophages (2, 3, 5).

Our data reveal previously unknown roles for IL-21–induced SOCS3 in promoting B cell proliferation and negatively regulating IgE switching. In vitro stimulation of B cells with the T cell co-stimulatory factors CD40L, IL-4, and IL-21 produced fewer B cells after 4 d when SOCS3 was absent. Although this indicated that early GC B cell proliferation might be impaired in the absence of SOCS3, there was no effect of SOCS3 deletion on either the cell cycle status of ex vivo GC B cells on day 8 of the response or on in vivo GC B cell numbers on day 10.

Complete deletion of IL-21 in mice increases the levels of both total and Ag-specific IgE in serum (16, 28, 37), and STAT3 deficiency in humans causes hyper-IgE syndrome (27). As deletion of SOCS3 in B cells also increases the production of Ag-specific IgE, all three molecules appear to be negative regulators of IgE switching. Class-switch recombination to IgG is division linked (38); therefore, the effects of IL-21, STAT3, and SOCS3 on IgE production may be via limiting the small fraction of B cells that continue to proliferate and switch to IgE.

IL-21 reduces B cell proliferation and blocks excess IgE production in response to a T cell-dependent Ag (16, 28, 37). In the absence of SOCS3, GC B cells may be more sensitive to IL-21 signals, causing an increase in IgE plasma cell production and
a reduction in cell proliferation at the later stages of the GC reaction. Enhanced STAT3 signaling, which is associated with IgG1 switching (7), might cause a concomitant increase in the proportion of GC B cells switching to IgG1. On balance, these two factors could result in an apparently normal number of IgG1-memory B cells and plasma cells exiting the GC in the absence of SOCS3.

Post-GC plasma cell formation requires STAT3 (7), which we have shown to be activated when plasma cells are exposed to IL-6. SOCS3 has a nonredundant role in regulating this pathway as its loss increases the duration of STAT3 phosphorylation. In addition, it has been shown that excess IL-6 signaling promotes a surplus of plasma cells (39–41). Alterations at the biochemical level, however, do not translate to cellular changes in SOCS3-deficient plasma cells in the steady state. The number of plasma cells in the spleen and BM was not affected by SOCS3 deficiency, and the general IgG1 plasma cell response to NP-KLH immunization was not substantially altered by the loss of SOCS3. The production of Ag-specific IgM, however, was significantly lower when SOCS3 was absent. Whether this can be attributed to a role for SOCS3 in sustaining the proliferation of B cells destined for extrafollicular plasmablasts responses or in inhibiting switching away from IgM is unclear.

By deleting SOCS3 exclusively in B cells, we have demonstrated the cell-intrinsic roles of SOCS3 in B cell and plasma cell development and behavior. Although SOCS3 was induced in B cells and plasma cells with IL-21 and IL-6 stimulation, respectively, its deletion did not have major cellular consequences in the steady state. Upon immunization with a T cell-dependent Ag, however, the maintenance of SOCS3-deficient GC B cells was impaired. A novel role for SOCS3 was found in the formation of Ag-specific IgM, however, was significantly lower when SOCS3 was absent. Whether this can be attributed to a role for SOCS3 in the cell-intrinsic roles of SOCS3 in B cell and plasma cell development.

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Disclosures

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References


