SOCS3 Targets Siglec 7 for Proteasomal Degradation and Blocks Siglec 7-mediated Responses

CD33-related Siglecs (sialic acid-binding immunoglobulin-like lectins) 5–11 are inhibitory receptors that contain a membrane proximal ITIM (immunoreceptor tyrosine-based inhibitory motif) (I/V/L)/XX(Y/L)/V, which can recruit SHP-1/2. However, little is known about the regulation of these receptors. SOCS3 (suppressor of cytokine signaling 3) is up-regulated during inflammation and competes with SHP-1/2 for binding to ITIM-like motifs on various cytokine receptors resulting in inhibition of signaling. We show that SOCS3 binds the phosphorylated ITIM of Siglec 7 and targets it for proteasomal-mediated degradation, suggesting that Siglec 7 is a novel SOCS target. Following ligation, the ECS E3 ligase is recruited by SOCS3 to target Siglec 7 for proteasomal degradation, and SOCS3 expression is decreased concomitantly. In addition, we found that SOCS3 expression blocks Siglec 7-mediated inhibition of cytokine-induced proliferation. This is the first time that a SOCS protein and that inhibitory receptors have been shown to be degraded in this way. This may be a mechanism by which the inflammatory response is potentiated during infection.

The Siglec2 family of receptors consists of sialoadhesin (Siglec 1/Sn), CD22 (Siglec 2), CD33 (Siglec 3), myelin-associated glycoprotein (MAG or Siglec 4), and Siglecs 5–11. CD33-related Siglecs (Siglecs 5–11) are characterized by an N-terminal V-set Ig domain that mediates sialic acid binding and varying numbers of C2-set Ig domains. Sialic acids are a family of 9-carbon sugars that are derivatives of neuraminic acid or ketodeoxynonulosonic acid. The CD33-related Siglecs contain an ITIM and an ITSM in their cytoplasmic tails (1). The ITIM consists of a tyrosine with a leucine or valine at the +3 position and a hydrophobic base at the −2 position ((I/L/V)/XX(Y/L)/V), where X denotes any amino acid. The ITIM tyrosine is phosphorylated by Src family protein tyrosine kinases followed by recruitment of SH2-containing phosphatases such as the inositol polyphosphate 5-phosphatase, SHIP, and protein tyrosine phosphatases SHP-1 and SHP-2 to inhibit signaling (2).

Suppressor of cytokine signaling (SOCS) molecules inhibit the JAK/STAT pathway, ensuring that cytokine responses are regulated. SOCS proteins (CIS and SOCS1–7) contain an SH2 domain and a SOCS box, which binds Elongin B/C and Cul5/Rbx1 (2). This complex can act as an E3 ligase and degrade associated proteins via the 26 S proteasome (9, 10). SOCS proteins have been shown to target JAK2 and EpoR for proteasome-mediated degradation (8). The SH2 domain of SOCS3 displays 39 and 41% homology with the N-terminal SH2 domains of SHP-1 and SHP-2, respectively. SOCS3 bound to the same ITIM-like sites as SHP-1/2 on gp130, LRH, and EpoR, indicating that SHP-1/2 and SOCS3 share a similar ligand binding specificity (11). In this study we found that SOCS3 interacts with Siglec 7 and regulates its turnover. We have shown here that SOCS3 binds to the phosphorylated ITIM of Siglec 7 and targets it to the proteasome for degradation, thereby blocking the inhibitory effects of Siglec 7 on cytokine-induced proliferation.

EXPERIMENTAL PROCEDURES

Constructs and Cells—Siglec 7 wild-type (WT) was cloned into pME185-FLAG and pMX-IRE5-EGFP vectors. Siglec 7Y341F pMX-IRE5-EGFP was created using QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). Rbx-1, Cul-5 (9), SOCS3, Elongin C, and Elongin B constructs were transfected into 293T cells as described previously (12). Ba/F3, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; E3, ubiquitin-protein isopeptide ligase; SOCS, suppressor of cytokine signaling; JAK, Janus kinase; STAT, signal transducers and activators of transcription; SH2, Src homology 2; EGFP, enhanced green fluorescent protein; IL-3, interleukin 3; GST, glutathione S-transferase; EV, empty vector; Tet, tetracycline.
Ba/F3-SOCS3 (13), SOCS3−/−, and WT MEF cells were retrovirally infected with Siglec 7 pMX-IRES-EGFP as described previously (14).

Siglec 7 Cross-linking—The cells were incubated with 10 μg/ml Siglec 7 monoclonal antibody (QA79) (Dr. L. Moretta, Istituto G. Gaslini, Genova, Italy) in serum free medium for 20 min at room temperature followed by incubation with goat anti-mouse (GAM) whole molecule (IgG) (20 μg/ml) for the indicated times.

Immunoprecipitations and Western Blotting—Cells were lysed on ice in 1× radioimmune precipitation lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.25% Na-deoxycholate, 1 mM EDTA, 1 mM Na3VO4, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride) followed by immunoprecipitation with anti-FLAG (M2) (Sigma) or anti-SOCS3 (Fusion Antibodies, Belfast, UK) bound to protein A-Sepharose beads. For GST pulldowns, lysates were incubated with 5 μg of SOCS3 GST and control GST fusion proteins (Fusion Antibodies) bound to streptavidin beads for 4 h. For peptide pulldowns, lysates were incubated with 10 μg of peptide bound to streptavidin-agarose beads for 4 h. Immunoprecipitates were analyzed by Western blotting with anti-FLAG, anti-His, anti-Myc, and γ-tubulin (Sigma), anti-SHP-2, and anti-SOCS3 (M20) (Santa Cruz Biotechnology, Santa Cruz, CA), anti-STAT5B (Dr. J. J. O’Shea, National Institutes of Health, Bethesda, MD), and anti-GST (Fusion Antibodies).

Proliferation Assays—Siglec 7 stable Ba/F3-SOCS3 cells were seeded at a density of 1 × 10⁵/ml and grown in RPMI 1640 medium containing 5% fetal calf serum and 5 units/ml IL-3 tetracycline (4 μg/ml) for 48 h. Fresh antibody was added at 24 h, and viable cells were measured by trypan blue exclusion and MTT assays.

RESULTS

SOCS3 Binds to the Phosphorylated ITIM of Siglec 7—Siglec 7 has been shown to recruit SHP-1/2 following pervanadate treatment (15). Cytokine receptors such as gp130 and the leptin and Epo receptors also recruit SHP-1/2 and SOCS3 to ITIM-like motifs, which contain a hydrophobic residue at position Y − 2 and a valine or leucine residue at Y + 3 (11). This suggests that SHP-1/2 and SOCS3 share a similar ligand binding specificity. Therefore, the SOCS3-GST fusion protein was used to examine any interaction with Siglec 7. 293T cells were transiently transfected with either 2 μg of empty vector (EV) or Siglec 7 pME18S-FLAG. Cell lysates were incubated with either GST or SOCS3-GST fusion protein bound to streptavidin beads for 4 h or immunoprecipitated with α-FLAG. Siglec 7 bound to the SOCS3-GST fusion protein (Fig. 1A, panel 1 (top), lane 4), whereas binding to the control GST fusion protein was undetectable (lane 2). The experiment consistently indicated an interaction between Siglec 7 and SOCS3.

To map the location of this interaction, phosphorylated and unphosphorylated Siglec 7 ITIM biotinylated peptides were used to pull down SOCS3. 293T cells were transiently transfected with EV or SOCS3 pME18S-FLAG, and lysates were incubated for 4 h with the peptides or immunoprecipitated with α-FLAG. SOCS3 associated with the phosphorylated ITIM peptide (Fig. 1B, panel 1, lane 5), whereas no association was observed with the unphosphorylated ITIM or the T cell receptor ITAM peptides (lanes 4 and 6). Endogenous SHP-2 was also associated with the phosphorylated ITIM peptide (Fig. 1B, panel 3, lanes 2 and 5). The data indicated that SOCS3 bound to the Siglec 7 ITIM in a phosphotyrosine-dependent manner.

Cell Surface Cross-linking of Siglec 7 in the Presence and Absence of SOCS3—Cross-linking results in activation of Siglec 7 by recruiting SHP-1/2 (15). Siglec 7WT and Siglec 7Y341F stable Ba/F3 cells (1 × 10⁷ cells/cell) were incubated with α-Siglec 7 (QA79) for 20 min at 37°C to determine the importance of the ITIM tyrosine residue. The cells were lysed and immunoprecipitated with α-FLAG. Cross-linking Siglec 7 resulted in a slight decrease in Siglec 7 expression (Fig. 2A, panel 1, lane 4), whereas cross-
linking did not affect Siglec 7Y341F expression (lane 8). Whole cell lysates were immunoblotted with α-STAT5B to confirm equal loading (Fig. 2A, panel 2). Degradation of Siglec 7Y341F was not observed, which indicated that the proximal tyrosine motif was important for the degradation of Siglec 7 in the absence of SOCS3. Loss of expression of Siglec 7 in lane 4 (Fig. 2A, panel 1) was consistent over three experiments; however, it was not statistically significant in the absence of SOCS3 according to the Students t test.

SOCS proteins form E3 ligase complexes and can target a range of proteins such as the growth hormone receptor, JAK2, Vav, and IRS1/2 for proteasomal degradation (8). As we had demonstrated that SOCS3 bound to the phosphorylated ITIM of Siglec 7 (Fig. 1), it was important to investigate whether SOCS3 affected the expression levels of Siglec 7. Therefore, a cross-linking experiment was performed in Siglec 7WT Ba/F3 cells with SOCS3 under a tetracycline-regulated promoter. Siglec 7WT Ba/F3-SOCS3 cells (1 × 10⁶ cells/pool) were incubated with α-Siglec 7 (QA79) for 20 min ± GAM for 5–15 min. The cells were lysed and treated as described previously.

Degradation of Siglec 7 and SOCS3 occurred in a time-dependent manner following cross-linking for 5, 10, and 15 min (Fig. 2B, panels 1 and 2, lanes 14–16, and supplemental Fig. 1), whereas in the absence of SOCS3 this degradation was not observed (Fig. 2B, panel 1, lanes 6–8). Therefore, we have demonstrated that 30 min of cross-linking resulted in a small decrease in Siglec 7 expression levels in the absence of SOCS3 (Fig. 2A, panel 1), whereas in the presence of SOCS3 cross-linking for 5 min was sufficient to initiate its degradation (Fig. 2B, panel 1) in correlation with the loss of SOCS3. The data imply that the Siglec 7 receptor is a novel target for SOCS3 and that both proteins are targeted for degradation together. This has not been reported previously for any SOCS target.

To further investigate this regulatory mechanism, SOCS3−/− cells were used to determine whether this protein was required for degradation of Siglec 7 to occur. Siglec 7 stable SOCS3−/− and WT MEFs (2 × 10⁶/pool) were incubated with α-Siglec 7 (QA79) for 20 min ± GAM for 30 min. The cells were lysed and treated as described previously. Cross-linking Siglec 7 for 30 min resulted in significant degradation of Siglec 7 (p < 0.0005) and SOCS3 (Fig. 2C, panels 1 and 2, lane 8), whereas minimal degradation was observed in the absence of SOCS3 (Fig. 2C, panel 1, lane 4). These data indicate that SOCS3 greatly enhances and accelerates turnover of Siglec 7 and that SOCS3 is degraded concomitantly. Induction of SOCS3 by lipopolysaccharide in peripheral blood mononuclear cells resulted in the down-regulation of surface expression of endogenous Siglec 7 (supplemental Fig. 2), implying that SOCS3 enhances its internalization and degradation.

SOCS proteins can act as E3 ligases by forming a complex with Elongin B/C and Cul5/Rbx1/2 (9, 10). SOCS3 has previously been shown to target IRS1/2 to the proteasome for degradation (16). To investigate whether Siglec 7 was degraded via the 26S proteasome, 293T cells were transiently transfected with EV and Siglec 7WT with the E3 ligase complex (Elongin B/C, Cul5, and Rbx1) ± SOCS3. The cells were treated ± proteasome inhibitors (MG132 and LLNL) for 30 min prior to

FIGURE 2. SOCS3 targets Siglec 7 for degradation. A, Siglec 7WT and Siglec 7Y341F stable Ba/F3 cells were incubated with α-Siglec 7 (QA79) ± GAM. Lysates were immunoprecipitated (IP) and immunoblotted (IB) with α-FLAG (panel 1). WCL was immunoblotted with α-STAT5B as a loading control (panel 2). Signal intensities of Siglec 7WT and Siglec 7Y341F expression levels were normalized to the signal intensity of STAT5B for triplicate experiments as illustrated (graph). B, stable Siglec 7WT Tet-regulated SOCS3 Ba/F3 cells were cultured ± Tet (4 μg/ml) for 48 h prior to incubation with α-Siglec 7 ± GAM. Lysates were immunoprecipitated and immunoblotted with α-FLAG (panels 1 and 2). WCL was immunoblotted with α-STAT5B as a loading control (panel 3). C, Siglec 7WT stable SOCS3−/− MEFs and WT MEFs were incubated with α-Siglec 7 ± GAM. Lysates were immunoprecipitated and immunoblotted with α-FLAG (panel 1). Lysates were immunoprecipitated with α-SOCS3 and immunoblotted with α-FLAG (panel 2). WCL was immunoblotted with γ-tubulin as a loading control (panel 3). Siglec 7WT expression was normalized to γ-tubulin for triplicate experiments (illustrated as a graph). **, p < 0.0005. D, 293T cells were transiently transfected with EV and Siglec 7WT with E3 ligase complex (Elongin B/C, Cul5, and Rbx1) ± SOCS3. Cells were treated with and without MG132 (0.5 μM) and LLNL (0.5 μM) prior to incubation with α-Siglec 7 ± GAM. Lysates were immunoprecipitated with α-FLAG and immunoblotted with α-FLAG (panels 1–3) and α-MyC (9E10) (panel 4). WCL was immunoblotted with α-MyC (panel 5). Signal intensities of Siglec 7 expression levels were normalized to the signal intensities of Elongin B for triplicate experiments as illustrated. **, p < 0.005.
incubation with α-Siglec 7 (QA79) for 20 min ± GAM for 15 min. The cells were lysed and treated as described previously.

Cross-linking for 15 min in the presence of the E3 ligase complex and SOCS3 resulted in significant Siglec 7 degradation (Fig. 2D, panel 1, lane 9) (p < 0.005) when compared with the absence of SOCS3 (lane 5). SOCS3 degradation occurred in correlation with Siglec 7 following cross-linking for 15 min (Fig. 2D, panel 2, lane 9). This degradation was rescued by pre-treatment with the proteasome inhibitors MG132 and LLNL (Fig. 2D, panel 1, lanes 14 and 18, and panel 2, lane 18). This suggests that after activation, SOCS3 may target Siglec 7 for proteasomal degradation. Degradation of Siglec 7 or SOCS3 was not observed when the E3 ligase complex was absent, indicating that this complex may be necessary for the degradation to occur in 293T cells (data not shown).

SOCS3 Inhibits Siglec 7-mediated Responses on Cytokine-induced Proliferation—Cross-linking CD33 and Siglec 7 has previously been shown to inhibit proliferation of chronic and acute myeloid leukemias (7, 17). Therefore, we decided to investigate whether engagement of Siglec 7 could inhibit proliferation of a cytokine-inducible cell line, and the effect of SOCS3 on this.

Siglec 7WT stable Ba/F3-SOCS3 cells were seeded ± Tet at 1 × 10⁵ cells/ml and cultured in RPMI medium supplemented with 5% fetal calf serum containing 5 units/ml of IL-3. Samples were cross-linked ± α-Siglec 7 (QA79) and GAM. F(ab’2) was also used to ensure that the observed effect was not due to the Fc portion of the cross-linking antibody, and similar results were obtained for GAM and F(ab’)2 (data not shown). Samples were analyzed by trypan blue exclusion assay. Cross-linking Siglec 7 in Siglec 7WT (Fig. 3B) cells in the absence of SOCS3 exhibited significant inhibition of IL-3-induced proliferation as determined by the Students t test (p < 0.005) compared with cells treated with GAM after 48 h. In the presence of SOCS3 no significant effect was observed. These observations suggest that engagement of Siglec 7 in the absence of SOCS3 inhibited IL-3-induced proliferation, whereas the presence of SOCS3 blocked this effect. The MTT assay was used to determine the function of the ITIM tyrosine and to confirm the results from the trypan blue exclusion assay. When compared with Siglec 7WT (p < 0.0005), Siglec 7Y341F (Fig. 3C) cells showed a much less significant effect on cytokine-induced proliferation in the absence of SOCS3 (p < 0.05), whereas the presence of SOCS3 blocked this effect. These data indicate a role for the ITIM of Siglec 7 in the inhibition of IL-3-induced proliferation. Similar results were obtained in a more physiological manner using Epo-Siglec 7 chimeras stimulated with Epo (data not shown). Engagement of Siglec 7WT inhibited cytokine-induced proliferation, possibly via SHP-1/2, and SOCS3 may compete with SHP-1/2 for binding to Siglec 7 leading to a block in inhibition.

**DISCUSSION**

Here we have demonstrated that SOCS3 can bind to the phosphorylated ITIM of Siglec 7 and target it for proteasomal-

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**FIGURE 3.** SOCS3 blocks effects of Siglec 7 engagement on cytokine-induced proliferation. A, Siglec 7WT stable Ba/F3-SOCS3 cells were cultured with and without Tet for 48 h. Lysates were immunoprecipitated (IP) and immunoblotted (IB) with α-FLAG (panel 1). WCL was immunoblotted with α-STAT5B as a loading control (panel 1). WCL was immunoblotted with α-STAT5B as a loading control (panel 1). B, Siglec 7WT stable Ba/F3-SOCS3 cells were seeded at 1 × 10⁵ cells/ml and cultured ± Tet. They were incubated with α-Siglec 7 (QA79) ± GAM for 48 h. Trypan blue exclusion assay determined the viability of the cells at 24-h intervals. Control and treated cells were all compared using Students t test to determine statistical significance (**, p < 0.005; ***, p < 0.0005). C, Siglec 7WT and Siglec 7Y341F stable Ba/F3-SOCS3 cells were cultured and cross-linked as described for B. 10 μl of MTT

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mediated degradation with the concurrent loss of SOCS3. This is the first time that degradation of SOCS3 has been observed in correlation with its target. We have also shown that SOCS3 overrides the inhibitory effect of Siglec 7 on cytokine-induced proliferation, which may be a novel means of regulating cytokine responses.

CD33-related Siglecs are expressed on cells of the innate immune system and bind sialylated compounds. These receptors contain an ITIM, which binds SHP-1/2 (15). The SH2 domain of SOCS3 bears 39 and 41% homology with the N-terminal SH2 domains of SHP-1 and SHP-2, respectively (11). Our data demonstrates that Siglec 7 can bind both SOCS3 and SHP-2 via the phosphorylated ITIM in the same manner as gp130 and the Epo and leptin receptors (11). These receptors competitively bind SOCS3 and SHP-1/2 to their ITIM-like motifs, suggesting that SOCS3 and SHP-1/2 may compete for binding to Siglec 7.

Stimulation by a wide range of cytokines induces SOCS family members, leading to inhibition of the JAK/STAT pathway in a negative feedback loop. SOCS proteins target EpoR, Vav, IRS1/2, and FAK for proteasomal degradation (8). We have identified Siglec 7 as a new target for SOCS3 resulting in proteasomal degradation of the Siglec 7-SOCS3 complex. Like HIV-1, Vif, and some F-box-containing proteins, SOCS3 may be degraded concurrently with its target by the 26 S proteasome (10). Degradation of any SOCS protein in complex with its target has not previously been shown. The SOCS box of SOCS proteins binds Elongin B/C and Cul5/Rbx1/2 to form an E3 ligase complex, which degrades associated proteins in a proteasome-dependent manner (9). We have shown that the E3 ligase complex enhances degradation of Siglec 7 and SOCS3. We propose that SOCS3 binds via its SH2 domain to the phosphorylated ITIM of Siglec 7, whereas the SOCS box binds other components of the E3 ligase complex resulting in degradation and thereby regulating the inhibitory response. Induction of SOCS3 by cytokines and bacterial products such as lipopolysaccharide and CpG-DNA (18, 19) can result in the degradation of Siglec 7 and SOCS3, implying an intricate means of regulating the inflammatory response.

Cross-linking CD33-related Siglecs causes inhibition of proliferation and induction of apoptosis (17, 20). We have demonstrated that engagement of Siglec 7 resulted in inhibition of proliferation of a cytokine-dependent cell line, whereas SOCS3 counteracted this effect, possibly because of proteasomal degradation of Siglec 7 and SOCS3. Our results indicate that apoptosis was not involved in Siglec 7-mediated inhibition (data not shown), suggesting the recruitment of SHP-1/2 similar to SIRP1α-mediated inhibition of hormone- and growth factor-induced proliferation (21).

Conditional gene knock-out of SOCS3 has demonstrated that SOCS3 is essential for regulation of gp130 and leptin receptor signaling, but unlike in the SOCS1−/− mice, no enhanced inflammatory response was observed (22–24). Our data imply that in the absence of SOCS3, Siglec 7 levels may be abnormally regulated, thereby leading to an aberrant inflammatory or cytokine response. SOCS3 has been implicated in the inhibition of inflammatory and autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis (25). SOCS3-mediated Siglec 7 degradation could be an important regulatory mechanism for controlling cytokine signaling during an inflammatory response.

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REFERENCES
1. Crocker, P. R., and Varik, A. (2001) Immunochemistry 103, 137–145
2. Billadeau, D. D., and Leibson, P. J. (2002) J. Clin. Investig. 109, 161–168
3. Deleted in proof
4. Falco, M., Biassoni, R., Bottino, C., Vitale, M., Sivori, S., Augugliaro, R., Moretta, L., and Moretta, A. (1999) J. Exp. Med. 190, 793–802
5. Mingari, M. C., Vitale, C., Romagnani, C., Falco, M., and Moretta, L. (2001) Immunol. Rev. 181, 260–268
6. Ikehara, Y., Ikehara, S. K., and Paulson, J. C. (2004) J. Biol. Chem. 279, 43117–43125
7. Vitale, C., Romagnani, C., Falco, M., Ponte, M., Vitale, M., Moretta, A., Bacigalupo, A., Moretta, L., and Mingari, M. C. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 15091–15096
8. Elliott, J., and Johnston, J. A. (2004) Trends Immunol. 25, 434–440
9. Kamura, T., Maenaka, K., Kotoshiba, S., Matsumoto, M., Kohda, D., Conaway, R. C., Conaway, J. W., and Nakayama, K. I. (2004) Genes Dev. 18, 3055–3065
10. Melehi, A., Goncalves, J., Santa-Martah, M., McPike, M., and Gabuzda, D. (2004) Genes Dev. 18, 2861–2866
11. Hortner, M., Nielsch, U., Mayr, I. M., Heinrich, P. C., and Haan, S. (2002) Eur. J. Biochem. 269, 2516–2526
12. Tannahill, G. M., Elliott, J., Barry, A. C., Hibbert, L., Calacano, N. A., and Johnston, J. A. (2005) Mol. Cell. Biol. 25, 9115–9126
13. Cohn, S. J., Sanden, D., Calacano, N. A., Yoshimura, A., Mui, A., Migone, T. S., and Johnston, J. A. (1999) Mol. Cell. Biol. 19, 4980–4988
14. Burrows, J. F., McGrattan, M. J., Rasle, A., Humbert, M., Baek, K. H., and Johnston, J. A. (2004) J. Biol. Chem. 279, 13993–14000
15. Avril, T., Floyd, H., Lopez, F., Vivier, E., and Crocker, P. R. (2004) J. Immunol. 173, 6841–6849
16. Rui, L., Yuan, M., Frantz, D., Shoelson, S., and White, M. F. (2002) J. Biol. Chem. 277, 42294–42298
17. Vitale, C., Romagnani, C., Puccetti, A., Olive, D., Costello, R., Chiossone, L., Pito, A., Bacigalupo, A., Moretta, L., and Mingari, M. C. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 5764–5769
18. Crespo, A., Filla, M. B., Russell, S. W., and Murphy, W. J. (2000) Biochem. J. 349, 99–104
19. Dalpke, A. H., Oppen, S., Zimmermann, S., and Heeg, K. (2001) J. Immunol. 166, 7082–7089
20. Nutku, E., Aizawa, H., Hudson, S. A., and Bochner, B. S. (2003) Blood 101, 5014–5020
21. Stofega, M. R., Wang, H., Ullrich, A., and Carter-Su, C. (1998) J. Biol. Chem. 273, 7112–7117
22. Johnston, J. A., and O’Shea, J. J. (2003) Nat. Immunol. 4, 507–509
23. Mori, H., Hanada, R., Hanada, T., Aki, D., Mashima, R., Nishinakamura, H., Torisu, T., Chien, K. R., Yasukawa, H., and Yoshimura, A. (2004) Nat. Med. 10, 739–743
24. Marine, J. C., Topham, D. J., McKay, C., Wang, D., Parganas, E., Stravopodis, D., Yoshimura, A., and Igle, J. N. (1999) Cell 98, 609–616
25. Suzuki, A., Hanada, T., Mitsuyama, K., et al. (2001) J. Exp. Med. 193, 471–481