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Interleukin-10 and cAMP-elevating agents cooperate to induce suppressor of cytokine signaling-3 via a protein kinase A-independent signal

Abstract

We have recently shown that IL-10 represents an efficient stimulus for suppressor of cytokine signalling (SOCS)-3 mRNA expression in human neutrophils and PBMC. Herein, we identify cAMP-elevating agents such as prostaglandin E2 (PGE2), PGE1, forskolin, dibutyryl cAMP (dbcAMP) and cholera toxin as a novel class of agonists able to induce SOCS-3 mRNA and protein

expression in human leukocytes, cooperating with interleukin-10 (IL-10) in such activities. While PGE2 or dbcAMP prolonged the stability of SOCS-3 mRNA isolated from IL-10-treated leukocytes, inhibitors of cAMP-dependent protein kinase A (H89, KT5720, and St-Ht31 peptide) did not influence the action of PGE2/dbcAMP and/or IL-10 on SOCS-3 mRNA and protein expression, implying that their effect are mediated through a PKA-independent pathway. Taken together, our data identify cAMP-elevating substances as a novel class of agonists able to trigger SOCS-3 expression, and suggest that SOCS-3 might be involved in the regulatory effects of cAMP-elevating substances.

Full text

INTRODUCTION

Despite the importance of negative control of cytokine signaling, to date, most of the efforts have been focused on the understanding of the mechanisms whereby cytokines exert their actions. However, a new family of suppressor of cytokine signaling (SOCS) proteins [also referred to CIS (for cytokine-induced Src homology 2) proteins] has been recently isolated, whose members are generating great interest [1-3]. These proteins have, in fact, the potential to negatively regulate the JAK (Janus kinase)-STAT (signal transducers and activators of transcription) signaling pathway [1-3]. For instance, CIS was shown to associate with the IL-3 and EPO receptors (IL-3R and EPOR) [4], and later shown to inhibit signaling through these receptors in an apparent classical negative feedback loop [5]. Likewise, biochemical characterization, as well as gene disruption studies, indicated that SOCS-1 is an important negative regulator of interferon-gamma signaling [6, 7]. Interestingly, although the most striking characteristic of the SOCS proteins is the induction of their expression *via* the JAK-STAT pathway in response to a wide range of cytokines, recent evidence indicates that there are exceptions to this notion. In particular, we and other researchers, have reported that both lipopolysaccharide (LPS) [8-10] and tumor necrosis factor-alpha (TNF-alpha) [10], which do not directly activate the JAK-STAT pathway, also represent efficient stimuli for SOCS-3 mRNA expression in neutrophils [8], monocytes [10], and murine macrophages [9, 10].

In this study, we investigated whether cyclic AMP (cAMP)-elevating agents could induce the expression of SOCS-3. cAMP is perhaps the best known and understood of the second messengers, and is generally considered to play a

suppressive role in immune regulation and mononuclear phagocyte functions [11]. Herein, we show that PGE_2 and other cAMP-elevating substances, including PGE_1, forskolin, and cholera toxin, as well as a stable cell-permeable cAMP analogue, dibutyryl cAMP (dbcAMP), not only directly stimulate the expression of SOCS-3 mRNA, both in human neutrophils and PBMC, but also cooperate with IL-10. We also show that SOCS-3 protein levels induced in PBMC by cAMP-elevating agents are synergistically augmented by costimulation of cells with IL-10. Finally, we demonstrate that inhibitors of cAMP-dependent protein kinase (PKA) do not influence the upmodulatory effects of cAMP-elevating substances and/or IL-10 on either SOCS-3 mRNA or SOCS-3 protein expression.

METHODS

Cell purification and culture

Peripheral blood mononuclear cells (PBMC) and highly purified granulocytes were isolated under LPS-free conditions from buffy coats of healthy donors as previously described [8, 12]. Immediately after purification, leukocytes were suspended in RPMI-1640 medium (Biowhittaker, Walkersville, MD, USA) supplemented with 10% low-endotoxin FCS (< 0.009 ng/ml, Seromed, Biochrom KG, Berlin, Germany), stimulated, plated in either polystyrene flasks (Biowhittaker) or six-well plates (Orange Scientific, Biowhittaker), and then cultured at 37° C, in a 5%, CO_2-atmosphere. Cells were routinely stimulated with 20 muM prostaglandin E_2 (PGE_2) (Sigma, St. Louis, MO, USA) in the presence or absence of 200 U/ml IL-10 (DNAX, Palo Alto, CA, USA) [8]. LPS (100 ng/ml from *E. coli*, serotype 026:B6, Sigma) was often used as a positive control for SOCS-3 induction [9, 10]. In other experiments, 20 muM PGE_1 (Sigma), 100 muM dbcAMP (Sigma), 1 mug/ml cholera toxin (CT) (Sigma), or 100 muM forskolin (Sigma), alone, or in combination with IL-10, were used. Theophylline (1 mM) (Sigma), a phosphodiesterase inhibitor, was always added to the cells 5 min prior to stimulation with cAMP-elevating substances. Preliminary experiments revealed that, at the concentration used, theophylline had no effect on SOCS-3 mRNA expression. In other experiments, leukocytes were pretreated for 30 min with 0.5-50 muM H89 (Alexis, San Diego, CA, USA), 5 muM KT5720, 10 muM St-Ht31 (inhibitor and control peptides, from Promega, Madison, WI, USA), 10 mug/ml polymyxin B sulphate (PMX), 10 mug/ml actinomycin D (Sigma), or neutralizing anti-hIL-10R2 mAb 1A8.3 [13], kindly provided by Dr. K.W. Moore (DNAX, Palo Alto, USA). At the times indicated in the results, cell-free supernatants were

harvested, whereas cell pellets were extracted for total RNA or lysed for Western blot analysis. All reagents were of the highest available grade and all buffers were prepared using pyrogen-free water for clinical use.

Cytokine measurements

Antigenic IL-8 and IL-10 were measured in the cell free-supernatants using specific ELISA developed with antibodies purchased from commercial sources: Euroclone (Devon, UK) for IL-8 and R&D System (Minneapolis, MN, USA) for IL-10. The detection limits of these ELISA were 20 pg/ml for IL-8 and 64 pg/ml for IL-10.

Northern blot analyses

Total RNA was extracted from PBMC or PMN by the guanidinium isothiocyanate method, processed for Northern blot and analyzed as already described [8, 12]. The extent of hybridization was quantitatively analyzed in an InstantImager (Packard Instruments, Meriden, CT, USA). The half-life of SOCS-3 mRNA under the various experimental conditions was calculated by regression analysis.

Immunoblots for SOCS-3

For the direct detection of SOCS-3, detergent lysates were prepared from PBMC according to the method previously described [8]. Briefly, after stimulation, cells were diluted in ice-cold PBS, centrifuged at 500 x g for 5 min at 4° C and resuspended in a lysis buffer [20 mM Hepes pH 7.9, 420 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% (v/v) Nonidet P-40, 20% (v/v) glycerol, 1 mM DTT], containing inhibitors of proteases (5 mug/ml leupeptin, 5 mug/ml pepstatin A, 1 mM PMSF, 1 mg/ml alpha1-antitrypsin), and phosphatases (1 mM Na_3VO_4, 10 mM NaF, 10 muM phenylarsin-oxide). Following a 20-min incubation on ice, cell debris was spun down (12,000 x g, 20 min, 4° C), and the supernatants frozen and stored at - 80° C. To avoid SOCS-3 degradation [14], 50 muM MG132 (N-CBZ-Leu-Leu-Leu-Ala, a proteosome inhibitor) (Sigma) were added to the cell cultures one hour prior to the end of incubation. Small aliquots of the various lysates were routinely processed for protein content determination, by using a protein assay kit (Bio-Rad, Hercules, CA, USA). Twenty-eight-40 mug of lysate were then diluted in sample buffer (40 mM Tris pH 6.8, 5% glycerol, 1% SDS, 1.25% beta-mercaptoethanol, and bromophenol-blue), and boiled for 5 min prior to electrophoretic separation on a 12% SDS-PAGE and subsequent transfer to

nitrocellulose by electroblotting [8]. To detect SOCS-3 protein (29 kDa MW), nitrocellulose membranes were incubated for 60 min at 4° C in TBS-Tween (150 mM NaCl, 20 mM Tris, 0.1% Tween-20 pH 7.5, 5% BSA), prior to an overnight incubation at 4° C with 1.4 mug/ml rabbit anti-SOCS-3 polyclonal antibodies (ImmunoBiological laboratories, Tokyo, Japan). Anti-SOCS-3 antibody binding was detected using horseradish peroxidase-conjugated anti-rabbit IgG (1:4000 dilution in TBS-tween) (Amersham Pharmacia Biotech), and revealed using the chemiluminescence system (ECL, Amersham Pharmacia Biotech), according to the manufacturer's instructions.

Intracellular cAMP assay

Freshly isolated PBMC (4×10^6 cells/ml) were incubated with 20 muM PGE_2 and/or 200 U/ml IL-10 for up to 60 min. Medium was then removed by centrifugation, and the cells lysed. Intracellular cAMP levels were measured according to the protocol provided with the Biotrak cAMP enzyme-immunoassay system kit (Amersham Pharmacia Biotech).

RESULTS

SOCS-3 mRNA and protein expression in human PBMC treated with PGE_2 or other cAMP-elevating substances in combination with IL-10

We have recently shown that IL-10 represents an efficient stimulus of SOCS-3 mRNA expression in human neutrophils and PBMC [8]. With the aim of identifying novel stimulatory conditions capable of inducing expression of SOCS-3, we examined the effect of PGE_2 and other cAMP-elevating substances, used either alone or in association with IL-10. Northern blot analysis shown in Figure 1 revealed that PGE_2, cholera toxin and forskolin, were all able to induce SOCS-3 mRNA in PBMC, although at levels lower than those induced by IL-10. PGE_2 and other cAMP-elevating substance-mediated increases in SOCS-3 mRNA steady-state levels were detectable as early as after 1 hour of stimulation, and remained stable for up to 6 hours (Figure 1). Remarkably, the effect of PGE_2, cholera toxin and forskolin, as well as that of dbcAMP, an analogue of cAMP (not shown), on SOCS-3 mRNA expression was synergistically potentiated by the simultaneous addition of IL-10. Maximal expression levels of SOCS-3 mRNA was reached by 1 hour, especially in the case of IL-10 plus PGE_2-treated cells (Figure

1). Identical patterns of SOCS-3 mRNA expression were observed in human neutrophils cultured as PBMC (data not shown).

Subsequently, we investigated whether human PBMC could also synthesize SOCS-3 protein, which, to date, has never been reported. Figure 2 shows that, although constitutively expressed in unstimulated PBMC at very low levels, SOCS-3 protein was markedly up-regulated by PGE_2, dbcAMP and IL-10. The ability of PGE_2 and dbcAMP to increase SOCS-3 protein was dose-dependent, with optimal results at 20 muM and 100 muM (Figure 2A), respectively, and was not inhibited by PMX (Figure 2B), under conditions in which this antibiotic effectively suppresses IL-8 production by LPS-stimulated PBMC (not shown). Furthermore, and in agreement with the Northern blot experiments, co-incubation of PBMC with IL-10 and PGE_2 or dbcAMP yielded a synergistic augmentation of SOCS-3 protein expression (Figures 3A and 3B). Such synergism was still evident at 21 hours of culture (Figure 3B), and occurred to a similar extent whether cAMP-elevating agents were applied prior to, or after IL-10 (not shown). Similar results were obtained in PBMC treated with PGE_1, cholera toxin or forskolin, cultured in the presence or absence of IL-10 (not shown).

The role of PKA in the induction of SOCS-3 by PGE_2 and/or IL-10 in PBMC

Since the activation of protein kinase A (PKA) has been shown to be an essential step in the induction of gene expression by cAMP-elevating substances [15], we investigated whether PGE_2 and other cAMP-elevating substances induce SOCS-3 expression *via* a PKA-dependent pathway. As shown in Figure 4, pretreatment of PBMC (or neutrophils, data not shown) with the PKA-selective inhibitor H89 [16, 17] did not influence the PGE_2 and/or IL-10-mediated induction of SOCS-3, either at the mRNA (Figure 4A), or protein (Figure 4B) level. Similar findings were observed when H89-pretreated PBMC were cultured with dbcAMP and/or IL-10. By contrast, H89 partially inhibited LPS-induced TNF-alpha mRNA expression in PBMC (Figure 4A), enhanced LPS-induced-IL-10 secretion (Figure 5A), and partially reversed the PGE_2 or dbcAMP-mediated inhibition of IL-8 production by LPS-stimulated PBMC or neutrophils (not shown) [18, 19]. Other PKA inhibitors, such as KT5720 (20) (Figure 4C) and St-Ht31 peptide [21] (not shown), did not inhibit SOCS-3 protein induction in PGE_2/dbcAMP and/or IL-10 stimulated PBMC either.

Possible involvement of endogenous IL-10 in the induction of SOCS-3 by PGE_2 and/or dbcAMP

cAMP-elevating substances have been demonstrated to rapidly up-regulate monocytic IL-10 mRNA expression and release *in vivo* and *in vitro* [22, 23], *via* activation of CREB-1 and ATF-1, and their binding to putative CRE elements in the upstream enhancer of the IL-10 promoter [24]. By inference, these observations raised the possibility that the various cAMP-elevating agents activate SOCS-3 gene expression simply *via* an autocrine effect of monocyte-derived IL-10, offering a plausible explanation for the synergistic action of PGE_2 and IL-10. However, the results shown in Figure 5 clearly exclude such a possibility. First of all, significant levels of IL-10 were measurable only in supernatants harvested from LPS- but not from PGE_2/dbcAMP-stimulated PBMC (Figure 5A). Secondly, the upmodulatory action of PGE_2/dbcAMP on SOCS-3 expression was not abrogated by the presence of anti-IL-10R neutralizing antibodies [13], whereas that mediated by IL-10 was completely suppressed (Figure 5B).

Measurement of cAMP in PBMC cultured with PGE_2 and/or IL-10

It is known that exposure of cells to PGE_2 results in a time-dependent increased production of intracellular second messenger cAMP. Therefore, we decided to determine whether IL-10 would potentiate the effect of PGE_2. Figure 6 shows that, at least at the concentrations used to induce SOCS-3, IL-10 had little influence on cAMP levels, either in untreated or in PGE_2-stimulated PBMC.

Effect of IL-10 and PGE_2 on SOCS-3 mRNA stability

In an attempt to elucidate the mechanisms whereby PGE_2 and IL-10 synergize in modulating the accumulation of SOCS-3 transcripts, we examined SOCS- 3 mRNA stability. PBMC were stimulated with IL-10 for 90 min, in the presence or absence of PGE_2, and then treated with actinomycin D to block the formation of additional transcripts. At increasing intervals thereafter, the cultures were processed for Northern blot analysis, and changes in the amounts of SOCS- 3 mRNA were quantified by InstantImager scanning. Figure 7 shows that PGE_2 prolonged the stability of SOCS-3 mRNA isolated from IL-10-treated PBMC. SOCS-3 mRNA half-lives in IL-10-, PGE_2-, and IL-10 plus PGE_2-stimulated cells were in fact 12 ± 1 min, 17 ± 3 min, and 25 ± 1 min, respectively. Similar results were obtained in IL-10 plus dbcAMP-treated PBMC (not shown).

DISCUSSION

The SOCS proteins belong to a new family of negative regulatory molecules that function in novel ways to suppress signal transduction pathways [1-3]. The findings presented here reveal that PGE_2 and other agents that increase cAMP, including PGE_1, forskolin, and cholera toxin, are able to upregulate SOCS- 3 mRNA steady-state levels in both PBMC and neutrophils, and SOCS-3 protein in PBMC. Similar results were obtained with dbcAMP, a very stable cell-permeable analogue of cAMP. To our knowledge, this constitutes the first demonstration of the ability of cAMP-elevating substances to modulate the gene expression of a SOCS family member, suggesting that induction of SOCS-3 might have a role in the biological activities exerted by PGE_2 and other agents that increase cAMP. Worthy of note is also the finding of a significant synergism between all cAMP-elevating substances used in this study and IL-10, resulting in a sustained upregulation of SOCS-3 mRNA and protein levels. Similarly, we previously reported a synergistic interaction between IL-10 and LPS in increasing SOCS-3 mRNA expression in neutrophils, regulated at the level of mRNA stability [8]. As in the latter experimental conditions [8], we have also demonstrated that the half-life of SOCS-3 transcripts is substantially prolonged in PBMC treated with IL-10 and/or PGE_2/dbcAMP, which is consistent with the notion that post-transcriptional events appear to be involved in the regulation of SOCS-3 mRNA accumulation.

Since it is well documented that elevation of cAMP results in the activation of cAMP-dependent protein kinase A (PKA), we evaluated the potential role of a cAMP/PKA pathway in regulating SOCS-3 expression by using structurally distinct PKA inhibitors, H89 [16, 17], KT5720 [20] and St-Ht31 [21]. Unexpectedly, these PKA inhibitors had no effect on SOCS-3 expression in PBMC and neutrophils exposed to cAMP-elevating agents and/or IL-10, even though they partially inhibited LPS-induced TNF-alpha mRNA expression in PBMC, enhanced LPS-induced-IL-10 secretion and reversed the inhibitory action of PGE_2/dbcAMP on IL-8 production in LPS-stimulated leukocytes. These results suggest that cAMP-dependent induction of SOCS-3 is PKA-independent. In this respect, it is well documented that PKA-independent pathways are involved in cAMP signaling mechanisms, in addition to PKA-dependent pathways. For instance, it has been shown that cAMP binds directly to some ion channels and to Rap1-specific GEF [25, 26]. In other studies, mitogenic signals initiated by cAMP have been shown to include both PKA-dependent pathways leading to p70s6k as well

as PKA-independent pathways regulating Akt and membrane ruffling [27]. Finally, PKA inhibitors have been shown to have no effect on cAMP-mediated inhibition of JAK3 expression in peripheral T cells exposed to PGE_2 [28], dbcAMP- and forskolin-induced suppression of con A-stimulated PBMC proliferation [29], or on the inhibitory action of PGE_2 on DNA synthesis in fibroblasts [30]. The nature of the signaling pathway triggered by PGE_2 and other cAMP-elevating agents leading to SOCS-3 induction remains however, unclear.

Our data also clearly establish that PKA activation has no role in mediating the synergism between IL-10 and the various cAMP-elevating agents. IL-10 was, in fact, not able to up-regulate the intracellular levels of cAMP, or to potentiate the stimulatory activity of PGE_2 on cAMP elevation. In addition, the availability of specific anti-IL-10R neutralizing antibodies allowed us to clearly exclude the possibility that the various cAMP-elevating agents activate SOCS-3 gene expression simply *via* an endogenous production of IL-10 [22, 23]. That the secretion of IL-10 from monocytes occurs through a cAMP/protein kinase A-dependent pathway, and, as such, is inhibited by H89 [23], and our present data indicate that SOCS-3 induction by PGE_2/dbcAMP is insensitive to H89, further supports the notion that the latter could not be mediated by endogenous IL-10. Thus, the molecular basis of the synergistic effect of cAMP-elevating substances and IL-10 on SOCS-3 synthesis remains an open question and needs to be elucidated.

We did not explore the biological significance of SOCS-3 induction by cAMP-elevating substances in combination or not with IL-10. cAMP controls many physiological cellular responses, including cell growth and differentiation [11, 15]. cAMP is also a potent modulator of the immune system, being able to either downregulate or upregulate expression of a number of cytokines. In general, signals provided by PGE_2, membrane-permeable stable analogues of cAMP, beta-adrenergic agonists, cholera toxin and phosphodiesterase inhibitors result in the downregulation of proinflammatory cytokines such as TNF-alpha, IFN-gamma or IL-12, and in the upregulation of anti-inflammatory cytokines, including IL-10 and IL-1Ra [11, 31]. These modulatory actions closely resemble those exerted by IL-10 [32]. Remarkably, additive effects of IL-10 and cAMP-elevating agents in the suppression of LPS-stimulated TNF-alpha synthesis have been recently reported [33]. Our preliminary experiments also revealed an additive effect betwen PGE_2 (or dbcAMP) and IL-10 in suppressing LPS-

stimulated IL-8 production in PMN. Although it has not been established yet whether SOCS-3 acts as an anti-inflammatory mediator induced by IL-10, taken together, our data reinforce the hypothesis that SOCS-3 may act as an intermediate molecule involved in the negative modulation of pro-inflammatory responses.

CONCLUSION

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References

1. Yasukawa H, Sasaki A, Yoshimura A. 2000. Negative regulation of cytokine signaling pathways. (Review) *Annu. Rev. Immunol.* 18: 143.

2. Naka T, Fujimoto M, Kishimoto T. 1999. Negative regulation of cytokine signaling: STAT-induced STAT inhibitor. (Review) *Trends Biochem. Sci.* 24: 394.

3. Krebs D L, Hilton D J. 2000. SOCS: physiological suppressors of cytokine signaling. (Review) *J. Cell Sci.* 113: 2813.

4. Yoshimura A, Ohkubo T, Kiguchi T, Jenkins N A, Gilbert D J, Copeland N G, Hara T, Miyajima A. 1995. A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin-3 and erythropoietin receptors. *EMBO J.* 14: 2816.

5. Matsumoto A, Masuhara M, Mitsui K, Yokouchi K, Ohtsubo M, Misawa H, Miyajima A, Yoshimura A. 1997. CIS, a cytokine inducible SH2 protein, is a target of the JAK-STAT5 pathway and modulates STAT5 activation. *Blood* 89: 3148.

6. Sakamoto H, Yasukawa H, Masuhara M, Tanimura S, Sasaki A, Yuge K, Ohtsubo M, Ohtsuka A, Fujita T, Ohta T, Furukawa Y, Iwase S, Yamada H, Yoshimura A. 1998.

A Janus kinase inhibitor, JAB, is an interferon-gamma-inducible gene and confers resistance to interferons. *Blood* 92: 1668.

7. Metcalf D. 1999. The SOCS-1 story. *Exp. Hematol.* 27: 1715.

8. Cassatella M A, Gasperini S, Bovolenta C, Calzetti F, Vollebregt M, Scapini P, Marchi M, Suzuki R, Suzuki A, Yoshimura A. 1999. Interleukin-10 (IL-10) selectively enhances CIS3/SOCS3 mRNA expression in human neutrophils: evidence for an IL-10-induced pathway that is independent of STAT protein activation. *Blood* 94: 2880.

9. Stoiber D, Kovarik P, Cohney S, Johnston J A, Steinlein P, Decker T. 1999. Lipopolysaccharide induces in macrophages the synthesis of the suppressor of cytokine signaling 3 and suppresses signal transduction in response to the activating factor IFN-gamma. *J. Immunol.* 163: 2640.

10. Bode J G, Nimmesgern A, Schmitz J, Schaper F, Schmitt M, Frisch W, Haussinger D, Heinrich P C, Graeve L. 1999. LPS and TNF-alpha induce SOCS3 mRNA and inhibit IL-6-induced activation of STAT3 in macrophages. *FEBS Lett.* 463: 365.

11. Zidek Z. 1999. Adenosine-cyclic AMP pathways and cytokine expression. (Review) *Eur. Cytokine Netw.* 10: 319.

12. Cassatella M A, Bazzoni F, Flynn R M, Dusi S, Trinchieri G, Rossi F. 1990. Molecular basis of interferon-gamma and lypopolysaccaride enhancement of phagocyte respiratory burst capability. Studies on the gene expression of several NADPH oxidase components. *J. Biol. Chem.* 265: 20241.

13. Crepaldi L, Gasperini S, Lapinet J A, Calzetti F, Pinardi C, Liu Y, Zurawsky S, de Waal Malefyt R, Moore K W, Cassatella M A. 2001. Up-regulation of IL-10R1 is required to render human neutrophils fully responsive to IL-10. *J. Immunol.* 167: 2312.

14. Cohney S J, Sanden D, Cacalano N A, Yoshimura A, Mui A, Migone T, Johnston J A. 1999. SOCS-3 is tyrosine phosphorylated in response to interleukin-2 and suppresses STAT5 phosphorylation and lymphocyte proliferation. *Mol. Cell. Biol.* 19: 4980.

15. Montminy M. 1997. Transcriptional regulation by cyclic AMP. (Review) *Annu. Rev. Biochem.* 66: 807.

16. Chijiwa T, Mishima A, Hagiwara M, Sano M, Hayashi K, Inoue T, Naito K, Toshioka T, Hidaka H. 1990. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H89), of PC12D pheochromocytoma cells. *J. Biol. Chem.* 265: 5267.

17. Ginty D D, Glowacka D, Bader D S, Hidaka H, Wagner J A. 1991. Induction of immediate early genes by $Ca2^{+}$ influx requires cAMP-dependent protein kinase in PC12 cells. *J. Biol. Chem.* 266: 17454.

18. Petrova T V, Akama K T, Van Eldik L J. 1999. Selective modulation of BV-2 microglial activation by prostaglandin E(2). Differential effects on endotoxin-stimulated cytokine induction. *J. Biol. Chem.* 274: 28823.

19. Au B T, Teixeira M M, Collins P D, Williams T J. 1998. Effect of PDE4 inhibitors on zymosan-induced IL-8 release from human neutrophils: synergism with prostanoids and salbutamol. *Br. J. Pharmacol.* 123: 1260.

20. Revan S, Montesinos M C, Naime D, Landau S, Cronstein B N. 1996. Adenosine A2 receptor occupancy regulates stimulated neutrophil function *via* activation of a serine/threonine protein phosphatase. *J. Biol. Chem.* 271: 17114.

21. Vijayaraghavan S, Goueli S A, Davey M P, Carr D W. 1997. Protein kinase A-anchoring inhibitor peptides arrest mammalian sperm motility. *J. Biol. Chem.* 272: 4747.

22. Platzer C, Meisel C, Vogt K, Platzer M, Volk H D. 1995. Up-regulation of monocytic IL-10 by tumor necrosis factor-alpha and cAMP elevating drugs. *Int Immunol.* 7: 517.

23. Woiciechowsky C, Asadullah K, Nestler D, Eberhardt B, Platzer C, Schöning B, Glöckner F, Lanksch W R, Volk H D, Döcke W D. 1998. Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced by brain injury. *Nat. Med.* 4: 808.

24. Platzer C, Fritsch E, Elsner T, Lehmann M H, Volk H D, Prosch S. 1999. Cyclic adenosine monophosphate-responsive elements are involved in the transcriptional activation of the human IL-10 gene in monocytic cells. *Eur. J. Immunol.* 29: 3098.

25. de Rooij J, Zwartkruis F J, Verheijen M H, Cool R H, Nijman S M, Wittinghofer A, Bos J L. 1998. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 396: 474.

26. Kawasaki H, Springett G M, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman D E, Graybiel A M. 1998. A family of cAMP-binding proteins that directly activate Rap1. *Science* 282: 2275.

27. Cass L A, Summers S A, Prendergast G V, Backer J M, Birnbaum M J, Meinkoth J L. 1999. Protein kinase A-dependent and -independent signaling pathways contribute to cyclic AMP-stimulated proliferation. *Mol. Cell. Biol.* 19: 5882.

28. Kolenko V, Rayman P, Roy B, Cathcart M K, O'Shea J, Tubbs R, Rybicki L, Bukowski R, Finke J. 1999. Downregulation of JAK3 protein levels in T lymphocytes by prostaglandin E2 and other cyclic adenosine monophosphate-elevating agents: impact on interleukin-2 receptor signaling pathway. *Blood* 93: 2308.

29. Bryce P J, Dascombe M J, Hutchinson I V. 1999. Immunomodulatory effects of pharmacological elevation of cyclic AMP in T lymphocytes proceed *via* a protein kinase A independent mechanism. *Immunopharmacology* 41: 139.

30. Arai H, Nomura Y, Kinoshita M, Nishimura F, Takigawa M, Takahashi K, Washio N, Takashiba S, Murayama Y. 1998. The inhibition of DNA synthesis by prostaglandin E2 in human gingival fibroblasts is independent of the cyclic AMP-protein kinase A signal transduction pathway. *J. Periodontal. Res.* 33: 33.

31. Feng W, Wang Y, Zhang J, Wang X, Li C, Chang Z. 2000. Effects of CTx and 8-bromo-cAMP on LPS-induced gene expression of cytokines in murine peritoneal macrophages. *Biochem. Biophys. Res. Commun.* 269: 570.

32. Moore K W, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann T R. 1993. Interleukin-10. (Review) *Annu. Rev. Immunol.* 11: 165.

33. Siegmund B, Eigler A, Moeller J, Greten T F, Hartmann G, Endres S. 1997. Suppression of tumor necrosis factor-alpha production by interleukin-10 is enhanced by cAMP-elevating agents. *Eur. J. Pharmacol.* 321: 231.

Figures

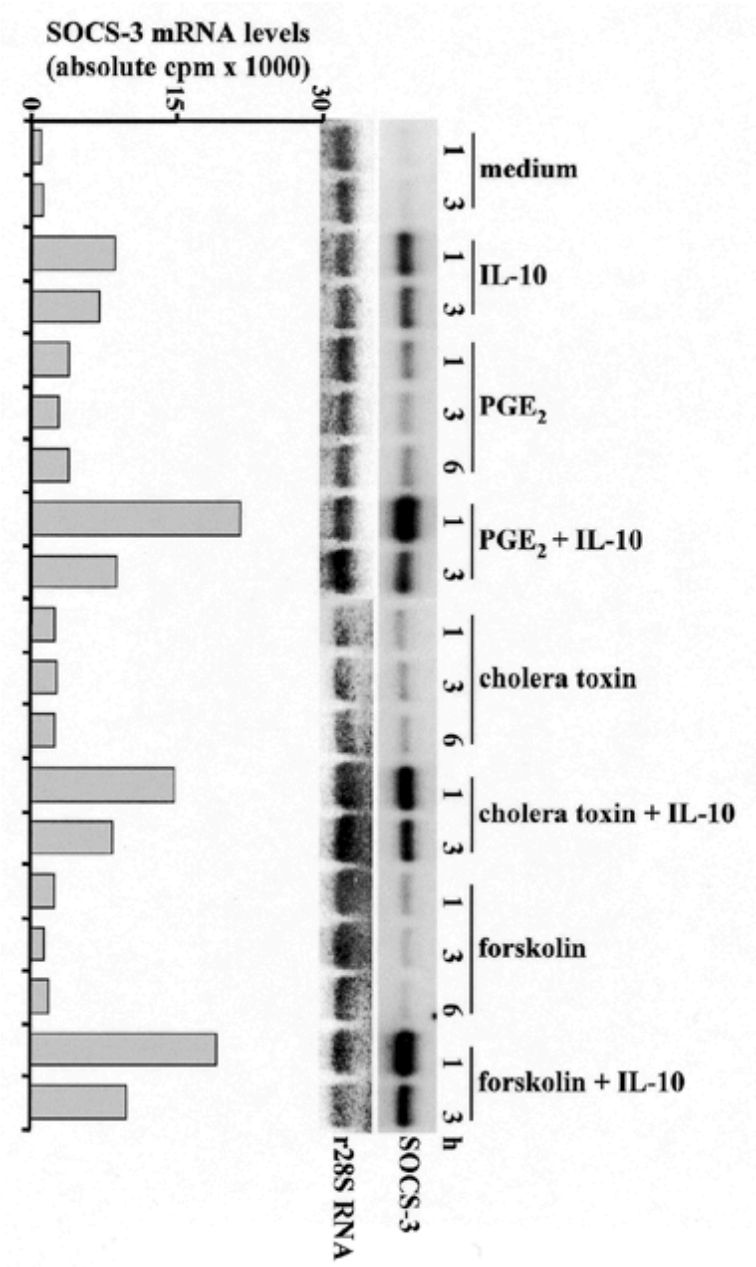
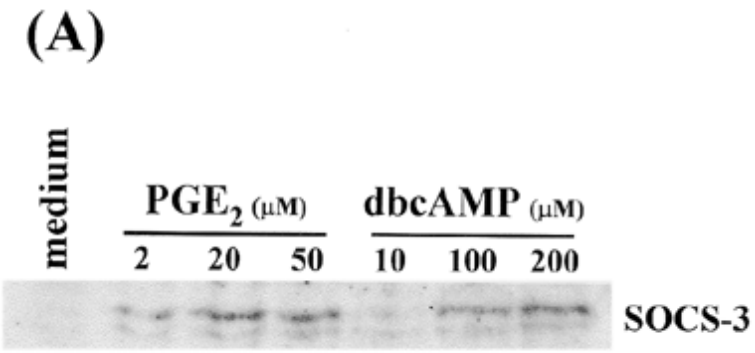


Figure 1
Time course of SOCS-3 mRNA expression in PBMC treated with various cAMP-elevating agents and/or IL-10. PBMC were incubated for the indicated times with 20 muM PGE_2, 1 mug/ml cholera toxin, and 100 muM forskolin in the presence or absence of 200 U/ml IL-10, before total

RNA extraction and Northern blot analysis for SOCS-3 mRNA expression. Ethidium bromide staining of r28S is shown in the lower part of the figure. The bottom panel shows a quantitative representation of SOCS-3 mRNA expression. This experiment is representative of three.



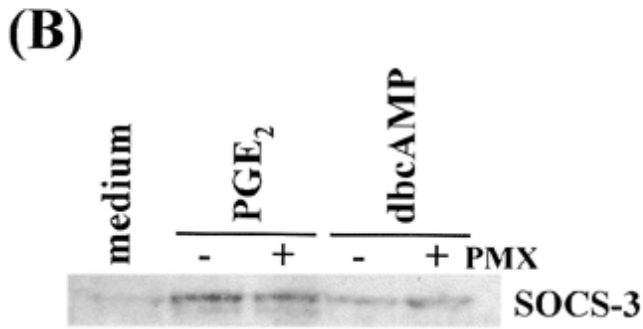


Figure 2
PGE_2 and dbcAMP genuinely upregulate SOCS-3 protein expression in PBMC. A: PBMC were treated for 2 hours with PGE_2 or dbcAMP, at different concentrations, prior to cell-lysis. **B:** PBMC were preincubated for 20 min with 10 mug/ml PMX and then stimulated for 2 hours with 20 muM PGE_2 or 100 muM dbcAMP. Thirty- five mug aliquots of PBMC lysates were loaded on the gels and immunoblots performed using specific anti-SOCS-3 antibodies.

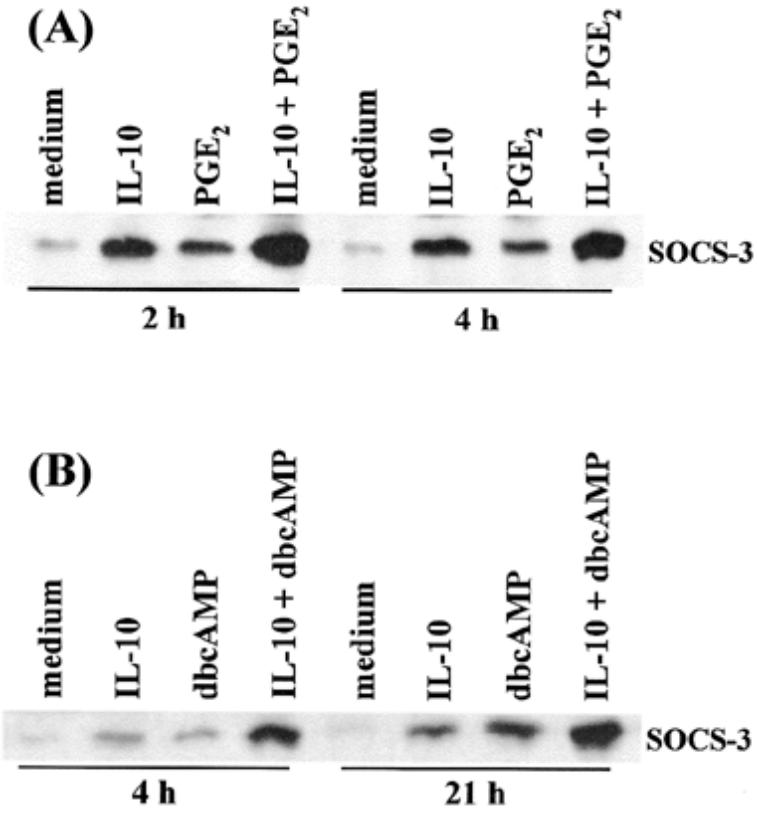


Figure 3

SOCS-3 protein expression in PBMC treated with PGE_2, dbcAMP, IL-10 and combinations thereof. PBMC were treated for the times indicated with 20 muM PGE_2 and/or 200 U/ml IL-10 **(A)**, or 100 muM dbcAMP and/or 200 U/ml IL-10 **(B)**, prior to cell-lysis. Twenty-eight mug aliquots of PBMC lysates were loaded on the gels, and immunoblots performed using specific anti-SOCS-3 antibodies. The data for each panel are representative of three independent experiments.

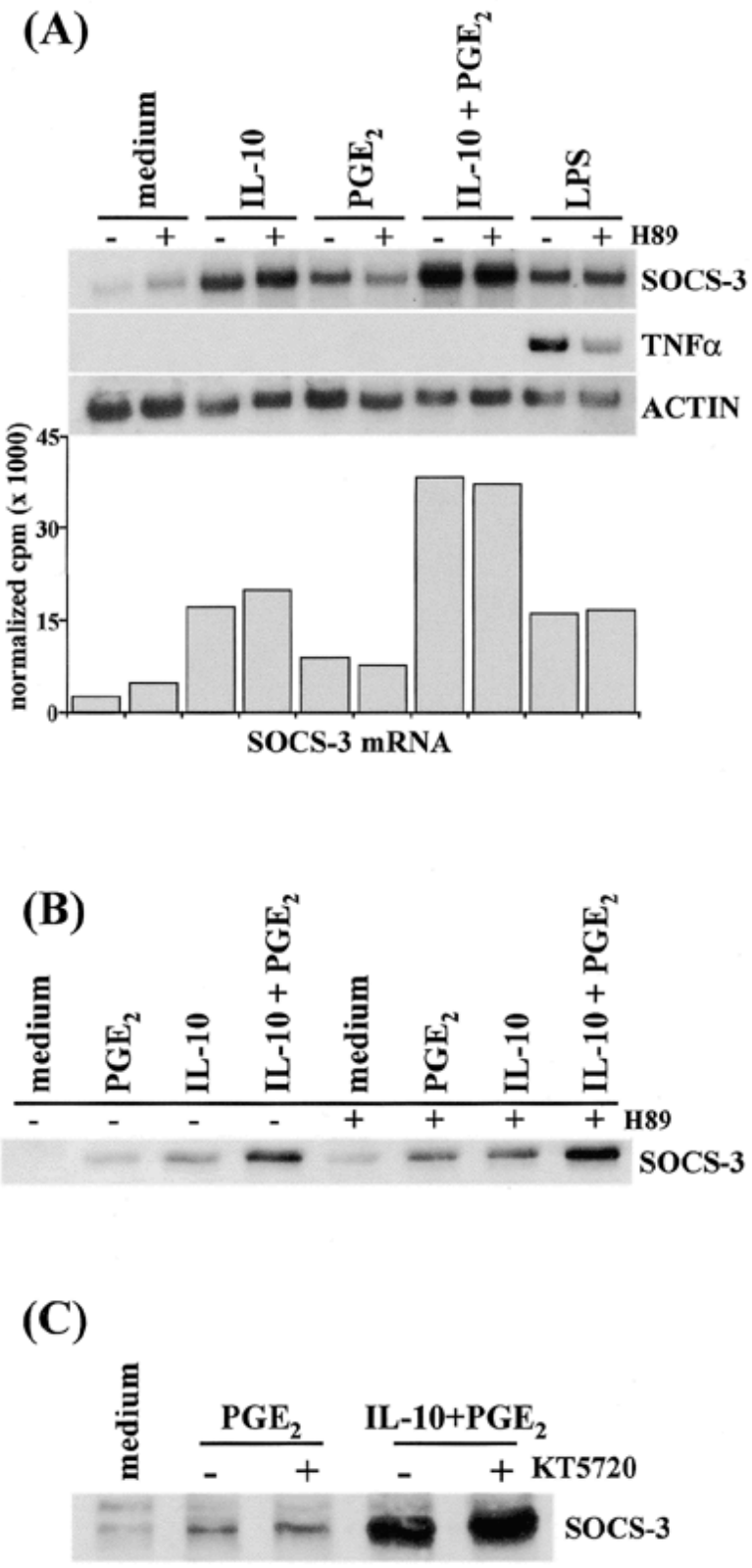


Figure 4

Effect of H89 on PGE_2 and/or IL-10-induced expression of SOCS-3. A: PBMC were preincubated for 30 min with 20 muM H89 and then stimulated for 2 hours with 100 ng/ml LPS, IL-10 and/or PGE_2. Total RNA was then

extracted and processed for Northern analysis using SOCS-3 and actin cDNAs. The lower panel shows a quantitative representation of SOCS-3 mRNA expression after actin normalization, by InstantImager analysis of the corresponding blots. Results are from one experiment representative of three. **B:** PBMC were preincubated for 30 min with 20 muM H89 and then stimulated for 3 hours with IL-10 and/or PGE_2, prior to cell-lysis. Thirty-five mug aliquots of PBMC lysates were loaded on the gels; and immunoblots performed using specific anti-SOCS-3 antibodies. Results are from one experiment representative of four. **C:** PBMC were preincubated for 30 min with 5 muM KT5720, and then stimulated for 2 hours with IL-10 and/or PGE_2. Forty mug aliquots of PBMC lysates were loaded on the gels, and immunoblots performed using specific anti-SOCS-3 antibodies.

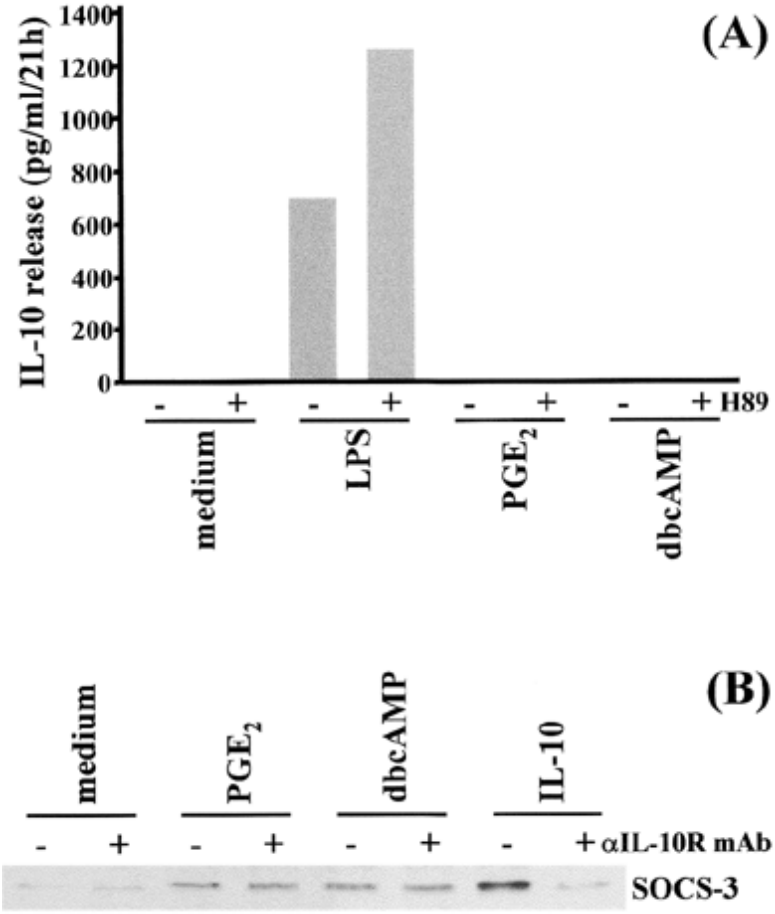


Figure 5

Endogenous IL-10 does not mediate the induction of SOCS-3 by PGE_2 and/or dbcAMP in PBMC.A: PBMC (3 x 10^6 cells/ml) were preincubated for 30 min with 20 muM H89 and then stimulated for up to 21 hours with 100 ng/ml LPS, 20 muM PGE_2, 100 muM dbcAMP, and combinations thereof. Cell-free supernatants were then collected and antigenic IL-10 levels determined by ELISA. **B:** PBMC were treated for 2 hours with 20 muM PGE_2, 100 muM dbcAMP and/or 200 U/ml IL-10 in the presence of neutralizing anti-IL-10R mAbs prior to lysis for characterization of SOCS-3 protein expression by immunoblotting.

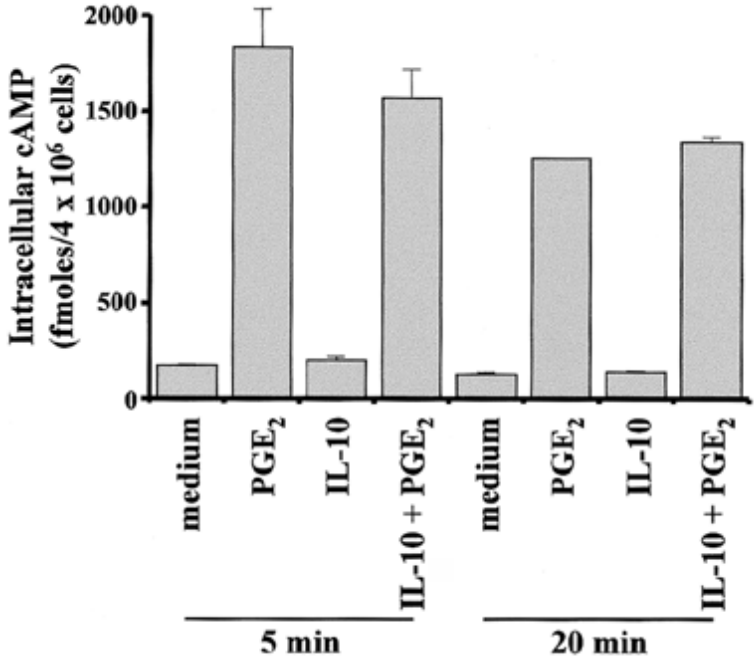


Figure 6

Effect of IL-10 on intracellular cAMP production in PGE_2-stimulated PBMC. PBMC (4 x 10^6 cells/ml) were cultured, for the times indicated, with 20 muM PGE_2, in the presence or absence of 200 U/ml IL-10, before measuring cAMP levels. This experiment is representative of three.

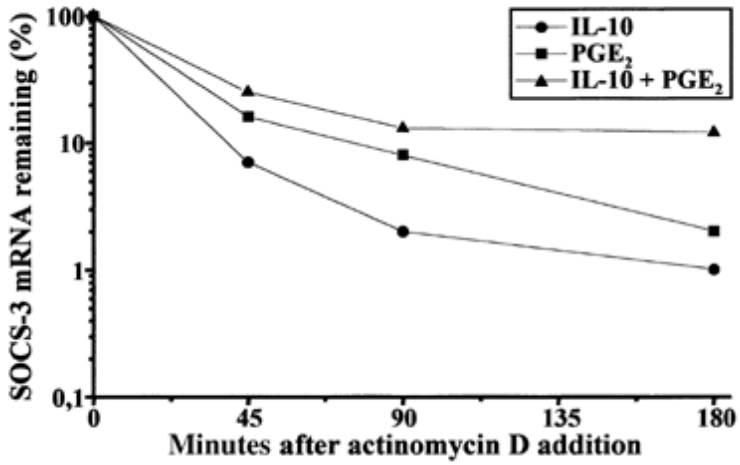


Figure 7
SOCS-3 mRNA turnover rate in PBMC stimulated with PGE_2 and/or IL-10. Quantitative representation of SOCS-3 mRNA decay. PBMC were cultured with 20 muM PGE_2, in the presence or absence of 200 U/ml IL-10. After 90 min. actinomycin D (5mug/ml) was added and the cells were further cultured for the indicated times. Total RNA was prepared and analyzed by Northern blotting with SOCS-3 and actin cDNA probes. The extent of hybridization was quantitatively analyzed and half lives were calculated by regression analysis.

Authors

Sara Gasperini, Luca Crepaldi, Federica Calzetti, Luana Gatto, Chiara Berlato, Flavia Bazzoni, Akihiko Yoshimura, Marco A. Cassatella

Dept. Pathology, Strada Le Grazie 4, I-37134 Verona, Italy.

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IL-1beta, TNF-alpha, GM-CSF, neuron, mRNA, protein.

Les éditions JLE

30 rue Berthollet, Bât. A,
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