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The levels of RAC3 expression are up regulated by TNF in the inflammatory response

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ABSTRACT

RAC3 is a coactivator of glucocorticoid receptor and nuclear factor-κB (NF-κB) that is usually overexpressed in tumors and which also has important functions in the immune system. We investigated the role of the inflammatory response in the control of RAC3 expression levels *in vivo* and *in vitro*. We found that inflammation regulates RAC3 levels. In mice, sub-lethal doses of lipopolysaccharide induce the increase of RAC3 in spleen and the administration of the synthetic anti-inflammatory glucocorticoid dexamethasone has a similar effect. However, the simultaneous treatment with both stimuli is mutually antagonistic. *In vitro* stimulation of the HEK293 cell line with tumor necrosis factor (TNF), one of the cytokines induced by lipopolysaccharide, also increases the levels of RAC3 mRNA and protein, which correlates with an enhanced transcription dependent on the RAC3 gene promoter. We found that binding of the transcription factor NF-κB to the RAC3 gene promoter could be responsible for these effects. Our results suggest that increase of RAC3 during the inflammatory response could be a molecular mechanism involved in the control of sensitivity to both pro- and anti-inflammatory stimuli in order to maintain the normal healthy course of the immune response.

1. Introduction

RAC3 (also known as SRC-3, AIB1, ACTR, p/CIP, TRAM-1) is a member of the p160 nuclear receptor coactivator family. The gene for this molecule was reported to be amplified in 5–10% of human breast tumors and the protein overexpressed in 60% of tumors, suggesting that RAC3 provides a growth advantage for breast cancer cells [1]. RAC3 is a bona fide oncogene and plays a role in initiation and progression of mammary gland tumorigenesis [34,40].

RAC3 was firstly identified as a coactivator for nuclear receptors [1,20,33], but it was later described as a coactivator for transcription factors which are structurally not related to the original targets. Such is the case of NF-κB [38] whose role in oncogenesis in addition to the immune response and several physiological functions have been described in detail [2,15,19]. Therefore, the investigation of the RAC3 role in tumors not dependent of steroid hormones, as well as additional functions not strictly related to nuclear receptors, is not surprising. In fact, most clinical studies have revealed the aberration of RAC3 in a broad spectrum of malignancies with high frequency, including pancreatic adenocarcinoma, hepatocellular carcinoma, gastric cancers, esophageal squamous cell carcinoma, and prostate cancer [14,16,18,31,37,40,42]. Moreover, there is strong evidence that some functions of RAC3 could be occurring through cytoplasmatic actions [8,27].

Abbreviations: Dex, dexamethasone; GC, glucocorticoids; GR, glucocorticoid receptor; HEK293, human embryonic kidney cells; LPS, lipopolysaccharide; NF-κB, nuclear factor kappa B; TNF, tumor necrosis factor

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Most of the RAC3 effects including the oncogenic role, are dependent of their expression levels and in a few cases, the gene amplification is the cause of its increase. Therefore, the molecular mechanisms that control the RAC3 levels deserve to be investigated. In that sense it was previously described that RAC3 gene is a target of E2F, being an E2F coactivator that promotes the cell cycle progression [26]. In addition, a posttranslational mechanism that controls its turnover was also described [22].

Although its oncogenic role appears to be more relevant, this nuclear receptor coactivator plays additional important functions in the control of growth and development involving cell proliferation, migration, differentiation, somatic growth, sexual maturation, female reproductive function, and vaso-protection [11,21,25,39]. More recently it was also described as an important mediator of the inflammatory response and septic shock [23,24].

The cytokines IL-1, IL-6 and TNF are the principal mediators of the inflammatory response but their over-induction and sustained biological activities are responsible for several undesirable effects, like septic shock and chronic disorders [4,9]. However, these inflammatory cytokines activate the hypothalamic–pituitary–adrenal axis (HPA) inducing the elevation of the systemic glucocorticoid levels [4,32]. These hormones are the most important anti-inflammatory and immunosuppressive agents that contain the overreaction of the immune system as well as autoimmune reactions through inhibition of cytokine gene expression and their biological actions [3,17].

RAC3 is a coactivator for the CG receptor (GR) and NF-κB. Therefore, in this work, we investigated if the inflammatory response may regulate the expression levels of RAC3 *in vivo* and *in vitro*.

2. Materials and methods

2.1. Cell culture and reagents

The human embryonic kidney HEK293 cells were cultured in DMEM (Gibco Laboratories, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen), penicillin (100 U/ml) and streptomycin (100 mg/ml). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. Unless stated otherwise, all reagents were obtained from Sigma Chemical Co., Invitrogen, Promega or Santa Cruz Biotechnology, USA. For all the experiments, the relative densitometric units (RDU) were determined from the original gel or picture using the NIH-Image J software.

2.2. In vivo model

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Male BALB/c mice, 6–8 weeks, weighing approximately 25–30 g, were purchased at the Buenos Aires University (Argentine). All mice were maintained in our animal facility with food and water at libitum for a minimum of 7 days before experimentation.

Mice were randomly divided into four groups: control group, LPS group (*Escherichia coli* 0111:B4-Sigma, 2 mg/kg, sublethal dose), dexamethasone (Dex, 6 mg/kg) and Dex plus LPS group. The group of Dex plus LPS was pre-treated with Dex 1 h prior intraperitoneal (i.p.) administration of LPS. The control group was treated i.p. with 200 μl of PBS. Animals were euthanized 6 or 24 h after LPS administration. The spleen was surgically removed and total RNA was extracted using TRIzol reagent (Invitrogen) following the manufacturer's instructions.

2.3. Expression vectors and reporter plasmids

The reporter plasmid containing a fragment of 2312 bp of the 5′ regulatory sequence of the RAC3 gene (−1970 to +335) upstream of Luciferase gene (PromRAC3-Luc), was previously described [13]. The RelA (pRc-RelA) and ssIκB (pRc-IκBss, carrying the mutated IκB at Ser32 and Ser36 to prevent phosphorylation and proteolysis) expression vectors were previously described [28]. The RAC3 siRNA (specific for RAC3 without effect over the others p160 family members), GRE-Luc and κB-Luc plasmids were previously described [13,38].

2.4. Luciferase assays

HEK293 cells were plated in 24-well plates 24 h prior to transfection at a density of 100,000 cells/well. Cells were transiently transfected with a total of 0.2 μg of DNA (including pRc-RelA and/or pRc-IκBss plus 20 ng of promRAC3-Luc or GRE-Luc or κB-Luc plasmids and 5 ng RSV-β Gal) using the calcium phosphate precipitation method as previously described [29]. The medium was replaced after 5 h and cells were stimulated with 500 nM of Dex and/or 10 ng/ml of human TNF 16 h later.

The assays for luciferase and β-Galactosidase activity were performed after 24 h of treatment using the appropriate substrates following the manufacturer's protocols (Promega Corp.). To achieve transfections with a constant amount of DNA, appropriate amounts of the parental empty expression vector were added to each well. In some experiments, cells were pre-incubated with NF-κB inhibitor Sulfasalazine (Sz) for 30 min before stimulation with TNF.

2.5. Real time PCR

Assays were performed as previously described [13]. Briefly, total RNA was isolated from HEK293 cells or spleen tissue by using the TRIzol protocol (Invitrogen). Reverse transcription was carried out by using the SuperScript II kit (Invitrogen) following the manufacturer's instructions. For gene expression analysis, qPCR was performed by using sequence-specific primers for: hRAC3 forward 5′-AAGTGAAGAGGGATCTGGA-3′ and reverse 5′- CAGATGACTACCA TTTGAGG-3′, and hGADPH forward 5′-TCTCCTCTGACTTCAACAGC-3′ and reverse 5′-GTTGTCATACCAGGAAATGA-3′ was used as an internal control.

To determine the effectiveness of the inflammatory response to LPS, spleen tissue IL-6, IL-1β and TNF mRNA levels were determined by qPCR [30]. It was performed using primers for: mIL-6 forward 5′-CCACTTCACAAGTCGGAGGCTTA-3′ and reverse 5′-GCAAGTGCATC ATCGTTGTTCATAC-3′, mIL-1 β forward 5′-CAACTGTGAAATGCCA CC-3′ and reverse 5′-GTGATACTGCCTGCCTGA-3′, mTNF-α forward 5′-AAGCCTGTAGCCCACGTCGTA-3′ and reverse 5′-GGCACCACTAGTT GGTTGTCTTTG-3′, and mRAC3 forward 5′-ACATGGTGCATATGAA-CAGC-3′ and reverse 5′-GATGTCAGCAGTATTTCTGATCG-3′. Specific mRNA expression levels were normalized to the housekeeping gene β-actin forward 5′-GCCAACCGTGAAAAGATGAC-3′ and reverse 5′-ACATGGCTGGGGTGTTGAA-3′, and the results are expressed as the fold change compared to control.

2.6. Western blot analysis

Western blot were performed as previously described [28]. Briefly, for RAC3 and RelA expression analysis, proteins were separated in an 8% SDS–PAGE. Protein samples were blotted to a PVDF membrane. After incubation with the corresponding primary and secondary antibodies, blots were developed by enhanced chemiluminescence (New England Nuclear, Boston, MA, USA).

2.7. Chromatin immunoprecipitation (ChIP)

After incubation for 45 min with 10 ng/ml of TNF, HEK293 cells were fixed with 1% of formaldehyde for 10 min and the ChIP was

performed as previously described [28]. In some experiments, cells were pre-incubated with the NF-κB inhibitor Sulfasalazine (SZ) for 30 min before stimulation with TNF.

Immunoprecipitation was performed using 25 μg of DNA in RIPA buffer with anti-Rel-A for 18 h at 4 °C in an orbital rocker. Immunoprecipitates were then incubated with Gamma Bind Sepharose-G protein (GE) for 2 h at 4 °C in an orbital rocker. After three washes, DNA was eluted from the pellets and proteins were digested with Proteinase K. Samples were heated with shaking at 65 °C for 4 h to reverse crosslinking. The extract containing DNA was precipitated with ethanol 70% and sodium acetate 0.3 M. Samples were resuspended in buffer TE and relative template enrichment after immunoprecipitation with respect to the input was determined by real time PCR using the primers 5′-TCCACAAATGTTAGGTATTACTGG-3′ and 5′-GGGAGCTCCCTCGGGATCCG-3′ that correspond to −199 κB elements of the RAC3 gene promoter. The specific signal was calculated as the difference between the mean value obtained from specific antibodies data and subsequently normalized to input values. Results were expressed as fold of increase relative to the input value.

3. Results

3.1. Inflammatory response up-regulates the RAC3 levels in the spleen

In order to analyze the effects of the inflammatory response and GC over the expression levels of RAC3, mice were treated with a sub-lethal dose of 2 mg/kg of LPS or 6 mg/kg of the synthetic glucocorticoid dexamethasone (Dex) or both simultaneously and the RAC3 mRNA expression was determined by qPCR in the spleen after 24 h.

As shown in Fig. 1A, this dose of LPS effectively induces an inflammatory response as determined by the expression of the inflammatory cytokines IL-1, IL-6 and TNF in the spleen. However, this response was significantly inhibited when Dex was administered 1 h before to LPS.

Fig. 1B shows that spleen from mice stimulated with LPS for 24 h have increased levels of mRNA for RAC3 as compared with the controls. Moreover, although GC plays an anti-inflammatory role, inhibiting the expression and activity of the inflammatory cytokines [3,17], Dex is a very good inducer of the RAC3 gene expression. However, the simultaneous treatment with both stimuli does not induce a synergic enhancement of RAC3 levels, but also, it results in a mutual antagonism, inhibiting the coactivator gene expression to basal levels.

3.2. TNF induces the increase of RAC3 gene expression

TNF is an inflammatory cytokine induced early in the inflammatory response that has multiple biological activities including its role in tumor development and metastasis [12,28,36].

Therefore, we investigated if TNF and Dex are capable of inducing the increase of RAC3 gene expression. For these experiments, we used the HEK293 cell line, which has receptors for TNF and GC. Similar to that observed *in vivo*, each one of these stimuli increases the expression levels of RAC3 mRNA (Fig. 2A) and protein (Fig. 2B), as determined by qPCR and western blot at 24 h after addition of TNF or Dex. However, the simultaneous treatment with both stimuli does not produce a mutual antagonism as observed between LPS and Dex.

In order to determine if TNF and Dex have a direct effect over the RAC3 gene promoter, HEK293 cells were transfected with a reporter plasmid containing luciferase under the control of a fragment of the RAC3 promoter (from −1970 to +335, containing putative target sequences for NF-κB binding) and stimulated with TNF, Dex or both together.

Fig. 2C shows that TNF or Dex increases the transcription dependent on the RAC3 promoter and, again, the simultaneous treatment with both stimuli does not produce a mutual antagonism, showing instead luciferase levels higher than when each one of these stimuli was applied separately.

3.3. NF-κB increases the transcription of RAC3 gene and could be a signal by which inflammatory cytokines upregulate the coactivator levels

The transcription factor NF-κB could be activated by multiple signals, being TNF one of them [15].

In order to determine if the effect of TNF over the RAC3 expression could be, at least partially mediated by NF-κB activation, we performed experiments using a specific inhibitor. We analyzed the levels of RAC3 mRNA or RAC3 gene promoter activity in HEK293 cells stimulated with TNF in the presence or not of Sulfasalazine (SZ), a potent and specific inhibitor of NF-κB activation [28,35].

Fig. 2A–C shows that SZ clearly inhibits the increase of RAC3 induced by TNF. Therefore, in order to confirm that NF-κB could be directly involved in the increase of both the RAC3 promoter activity and mRNA expression levels, we analyzed if this transcription factor is able to bind the putative NF-κB target sequence present in the RAC3 promoter fragment that we used in the reporter assays. Therefore, we performed ChIP experiments, where the Rel-A (the active subunit of the most abundant NF-κB dimer) was immunoprecipitated after TNF stimulus and the DNA bound to these complexes was amplified by qPCR using specific primers. Fig. 2D shows a significant increase on the NF-κB binding to this sequence in response to TNF, which was blocked by the addition of SZ.

In order to confirm the functional role of NF-κB on the RAC3 gene promoter, we performed additional experiments analyzing the effects of Rel-A. HEK293 cells were transfected with an expression vector for Rel-A, or the control empty vector and simultaneously, with or without the expression vector for the super-repressor IκB (ssIκB). This protein is unable to be phosphorylated and degraded, keeping NF-κB inactive. We analyzed the effect of Rel-A and/or ssIκB overexpression over RAC3 gene promoter activity, levels of RAC3 mRNA and proteins. Fig. 3 shows these results: the increase of Rel-A induces an enhanced transcriptional activity of the RAC3 gene promoter (Fig. 3A) giving increased levels of RAC3 mRNA (Fig. 3B) and RAC3 protein (Fig. 3C). The ssIκB overexpression significantly inhibited all these effects.

3.4. NF-κB and GR-mediated transactivation require RAC3 expression

In physiological conditions, most of the immune response involves the expression of NF-κB target genes while the anti-inflammatory effects require GR. In order to induce the expression of their target genes, GR and NF-κB uses several coactivators, like RAC3, SRC-1 and TIF-2, all members of the p160 family. However, is not clear whether there is a specific requirement for RAC3 for GR and NF-κB activity, or if other members could compensate for some RAC3 functions. Therefore, we investigated the impact of RAC3 depletion in the activity of GR, NF-κB and their mutual antagonism [38].

A low or undetectable expression pattern of p160 coactivators is usually found in normal tissues. On the other hand, HEK293 are non-tumoral cells that also express almost undetectable levels of these molecules [8]. Thus, this cell line is a good model to investigate the effect of RAC3 depletion on NF-κB and GR-dependent transcription, without the possible compensatory effect due to overexpression of the other p160 coactivators.

Therefore, cells were transfected with a RAC3 siRNA expression vector or the scrambled control. The NF-κB and GR transcriptional activity were analyzed by reporter assays using the κB-Luc or

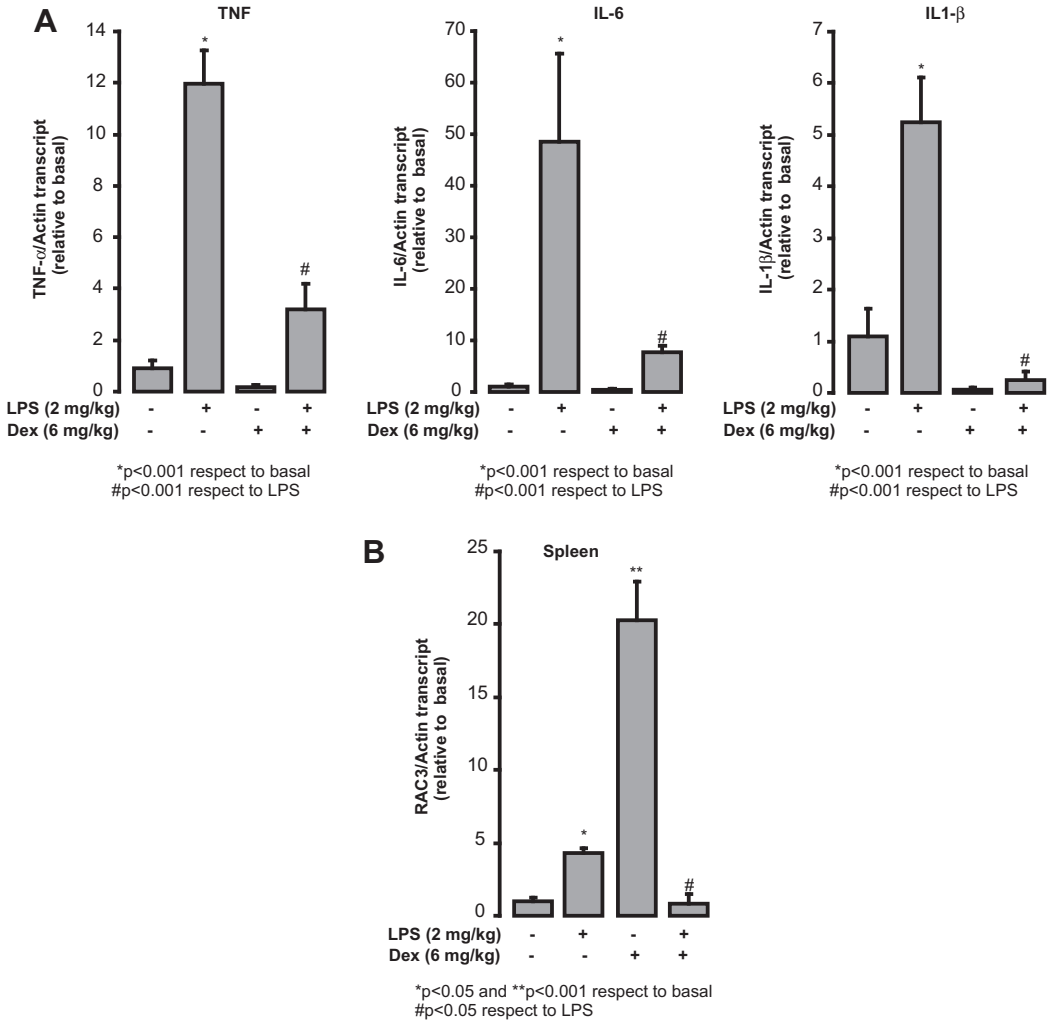

Fig. 1. Pro- and anti-inflammatory signals regulate the RAC3 expression: Balb/c mice were treated (ip) with LPS (2 mg/kg), Dex (6 mg/kg) or Dex plus LPS and sacrificed after 6 (A) or 24 h (B) of treatment. The mRNA levels of pro-inflammatory cytokines TNF, IL-6 and IL-1β (A) or RAC3 (B) from spleen were determined by qPCR, using specific primers. Diagram bars correspond to the average ± SD of five animals, normalized to Actin and expressed as a ratio respect to the control condition. Results were analyzed by Tukey's Test.

GRE-Luc reporter plasmids and cells were stimulated for 24 h with TNF (10 ng/ml), Dex (500 nM) or both simultaneously.

As shown in Fig. 4, under normal RAC3 gene expression and as expected, TNF induced the NF-κB -mediated transcription, which was inhibited by Dex (Fig. 4A) while Dex induced the GR-mediated transcription, that was inhibited by TNF (Fig. 4B) [38]. However, all NF-κB- and GR-dependent transcription was significantly inhibited when RAC3 expression was blocked (Fig. 4C, at amplified scale). Moreover, the mutual antagonism between both signals was not detected in the absence of RAC3 expression.

These results demonstrate that RAC3 is required for the NF-κB- and GR-mediated transcription, as well as for their mutual antagonism. Moreover, in non-tumoral cells, which have limiting quantities of p160 coactivators [8], RAC3 cannot be replaced by the other members. Therefore, their up regulation by inflammatory cytokines and GC could be the mechanism to ensure the normal course of the inflammatory response, which requires the NF-κB target gene expression, as well as some GR target genes.

4. Discussion

RAC3 is a coactivator of both GR and NF-κB [1,20,33], thus, the fact that inflammatory cytokines and GC could be regulating its expression levels is certainly no surprise.

In this work, we found that mice stimulated with LPS, at a dose that induces an inflammatory response was able to increase the expression levels of RAC3 in the spleen. We observed that this happens at transcriptional level. Similar results were obtained *in vitro* by stimulation with TNF, one of the inflammatory cytokines induced by LPS and the principal mediator of septic shock [5]. Although LPS or another signal induced by LPS in physiological conditions could be regulating the RAC3 levels, at least, TNF shows to be an efficient stimulus. Moreover, we found that this cytokine increases the RAC3 promoter activity. Interestingly, although TNF triggers several different intracellular signaling pathways, their effect was blocked by addition of the NF-κB specific inhibitor SZ, suggesting that this is the most important pathway to up regulate the coactivator. Moreover, NF-κB activation could be one of the signals by which the inflammatory response may increase the levels of RAC3. In this regard, we found that RAC3 promoter has a functional target sequence specific for NF-κB binding. In fact, this promoter responds to TNF stimulation or Rel-A overexpression in reporter assays and binding of the active transcription factor. Taken together, all these results suggest that NF-κB activation and binding to the RAC3 gene promoter leads to the up-regulation of this coactivator during the inflammatory response.

In a physiological context, as part of the natural control of the inflammatory response, the inflammatory cytokines activate the

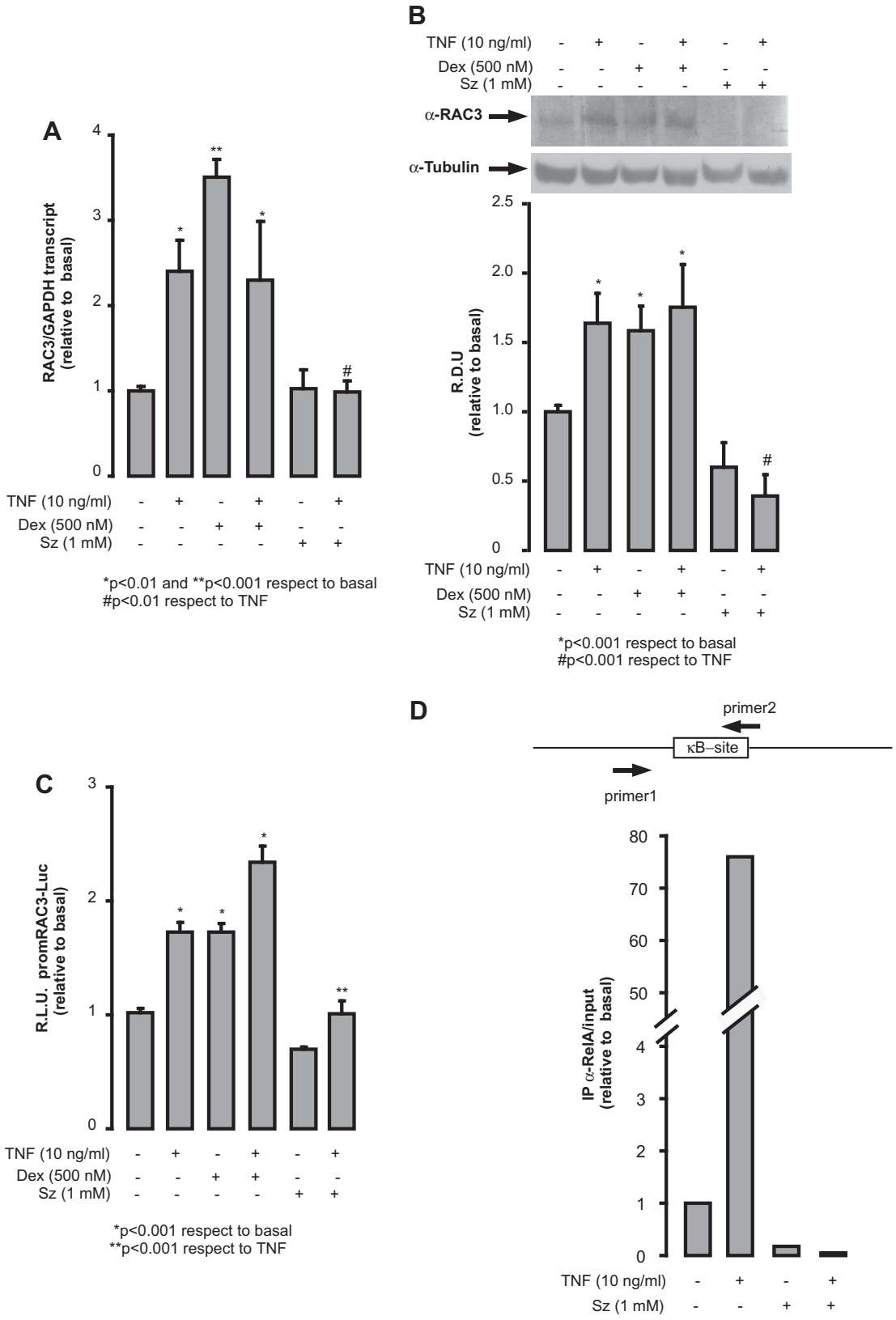

Fig. 2. TNF and dexamethasone induce the increase of RAC3 gene expression: HEK293 cells were stimulated for 24 h with TNF (10 ng/ml), Dex (0.5 mM) and Dex plus TNF in the presence or not of the NF-κB inhibitor Sz (1 mM). (A) RAC3 expression was determined by qPCR and diagram bars correspond to average ± SD of triplicate hRAC3, normalized to GAPDH and relativized to the control condition. (B) Protein levels were determined by Western blot. Diagram bars correspond to the average ± SD of five independent experiments expressed as the relative densitometric units (R.D.U.) determined respect to the Tubulin expression. (C) HEK293 cells transfected with the promRAC3-Luc plasmid were stimulated at similar experimental conditions as (A) and reporter activity was determined after 24 h. The diagram bars correspond to the average of relative light units (R.L.U.) normalized with the corresponding β-Galactosidase values. (D) HEK293 cells were stimulated with TNF (10 ng/ml) for 45 min in the presence or not of the Sz (1 mM), and ChIP assays were performed by immunoprecipitation with Rel-A antibody. Diagram bars correspond to the relative template enrichment after IP respect to the input. Upper diagram correspond to section of RAC3 gene promoter containing κB elements to be amplified by qPCR. In all cases, the results were analyzed by Tukey's Test.

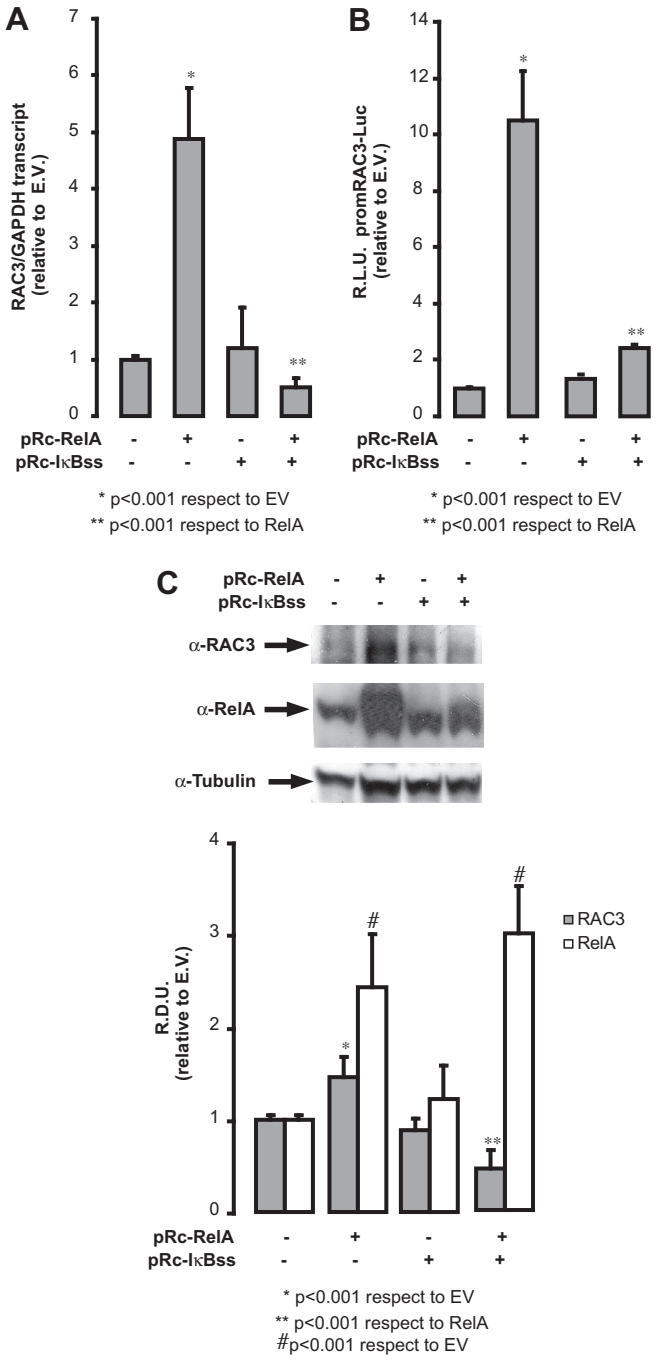

Fig. 3. NF-κB increases the RAC3 gene transcription: HEK293 cells were transfected with Rel-A expression vector or the empty control, in the presence or absence of the ssIκB expression vector. (A) RAC3 expression was determined by qPCR after 24 h post-transfection and diagram bars correspond to average of triplicate ± SD of mRNA hRAC3 normalized to GAPDH and relativized to the control condition. (B) HEK293 cells were transfected with the promRAC3-Luc plasmid; co-transfected at similar experimental conditions as (A) and reporter activity was determined after 24 h. The diagram bars correspond to the average of relative light units (R.L.U.) normalized with the corresponding β-Galactosidase values. (C) Protein levels were determined by Western blot and diagram bars correspond to average ± SD of relative densitometric units (R.D.U.) of three independent experiments, normalized respect to the Tubulin expression. In all cases, results were analyzed by Tukey's Test.

HPA axis inducing the increase of the anti-inflammatory GC in order avoid the immune overreaction [4,32]. Thus, the increase of RAC3 expression levels that was observed *in vivo* after 24 h of LPS administration, are probably the result of a balance in the crosstalk between the pro- and anti-inflammatory signals.

In order to determine the specific effect of GC over the RAC3 expression, we analyzed the action of Dex administered both *in vivo* and *in vitro*. In both cases, we found that the hormone induces the increase of RAC3 expression. Moreover, our results of qPCR and reporter assays suggest it could be happen at transcriptional level, perhaps through a direct action of the activated GR and binding to putative target sequences in the RAC3 gene promoter. However, our results obtained *in vivo* and *in vitro*, where Dex was administered together with LPS or TNF, suggest that additional mechanisms related to the crosstalk with inflammatory signals could not be excluded. In this regard, although each one of these stimuli were RAC3 up-regulatory signals, no synergism was observed *in vivo* or *in vitro*. Moreover, *in vivo* simultaneous stimulation with LPS plus Dex shown to be mutually antagonistic, given RAC3 mRNA levels similar to basal conditions. Meanwhile *in vitro*, all the stimuli, alone or combined, induced the increase of RAC3 promoter and gene expression. The differences between the promoter activity and gene expression could be explained perhaps, because the reporter assays were performed only with a fragment of the RAC3 promoter and additional regulatory sequences having a role in the control of RAC3 gene expression are not included. Concerning the antagonism, only observed *in vivo*, probably suggest that GC-mediated inhibition of inflammatory cytokines synthesis could be a limiting step for the increase of RAC3 expression. Moreover, in the physiological context, under LPS stimulation, the hormone was unable to enhance the RAC3 gene expression. However, the cause of the observed differences between *in vivo* and *in vitro* conditions and the precise molecular mechanism that are involved, remains to be determined, although the GR and NF-κB mutual antagonism appears to be the less probable [10,38].

It has been previously demonstrated that mice SRC-3$^{-/-}$ are more susceptible to infection in eye and skin [7,39] and markedly hypersensitive to LPS-induced endotoxic shock [6]. In agreement with previous reports, in response to LPS, SRC-3$^{-/-}$ macrophages produce significantly more pro-inflammatory cytokines such as TNF, IL-6, and IL-1β than wild-type controls. Although they express similar amounts of cytokine mRNAs, it was found that SRC-3 can exert effects at translational levels, acting as a translational repressor [41]. However, others have found that peritoneal macrophages of SRC-3-deficient mice showed a decrease in bacterial phagocytosis in culture and an increase in apoptosis, which was consistent with a defective bacterial clearance observed in SRC-3-deficient mice [6]. All together, these evidences strongly suggest an important role for RAC3 in the control of the immune response. Moreover, all the previous reports support the knowledge that a normal expression of this coactivator is required in order to ensure the normal course of the immune response. In normal physiological conditions, GC plays a central role avoiding the harmful effects of the immune overreaction and is the best anti-inflammatory control.

However, all this evidence arises from experiments performed in knockout animals, where some compensatory mechanisms could be developed during their life.

In agreement with our results concerning NF-κB and GR transcriptional activity (Fig. 4) in a cell line having low levels of p160 coactivators [8], the absence of RAC3 shows to be a key limiting factor for NF-κB and GR transactivation. This function could not be compensated, at least within 24 h, by the activity of another coactivator. Interestingly, the same low levels of coactivators could be found in normal mouse and human tissues, while the constitutive overexpression is related to tumoral cells.

Taken together, all our results suggest that, in normal conditions, the increase of RAC3 during inflammatory response could be a molecular mechanism that ensures the expression of NF-κB and GR target genes required in the normal healthy course of the immune and anti-inflammatory response.

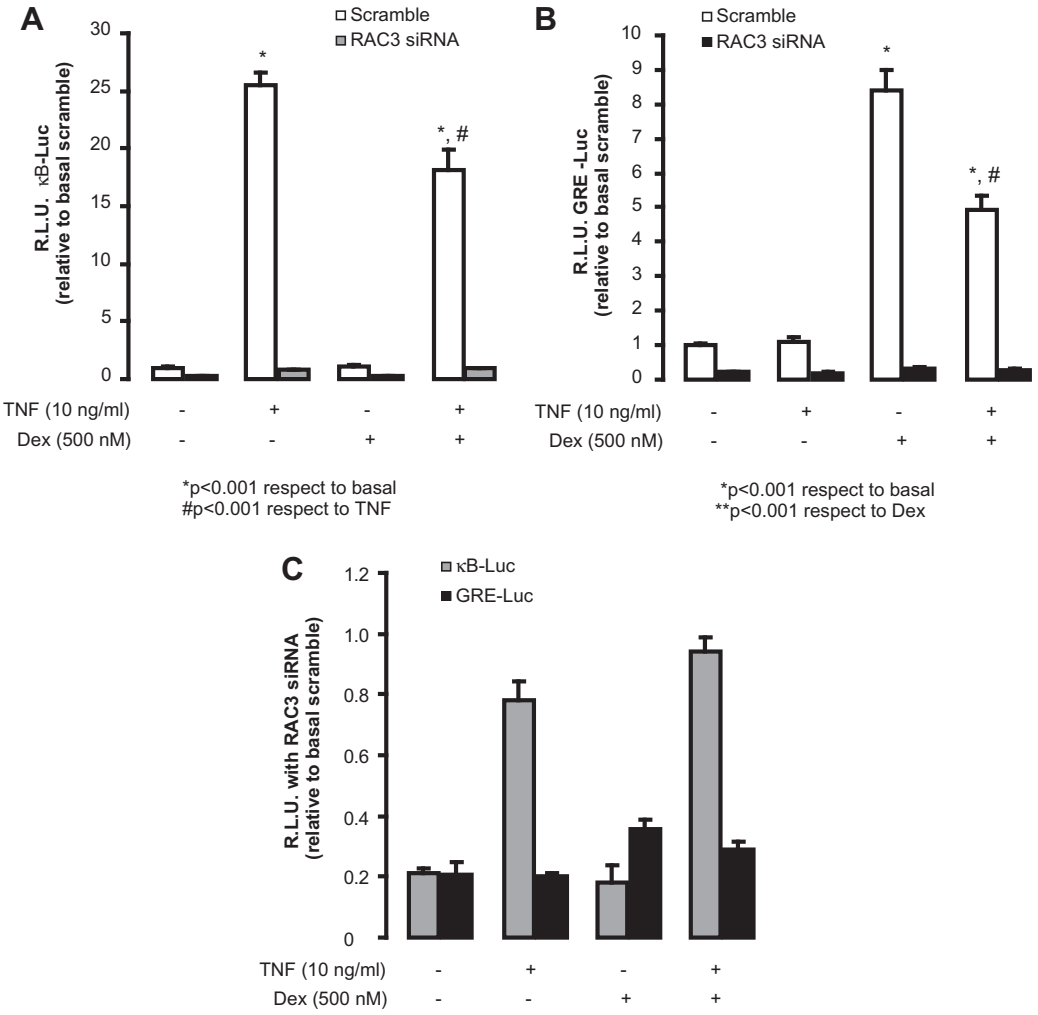

Fig. 4. RAC3 expression is required for NF-κB and GR-mediated transactivation: HEK293 cells transfected with RAC3 siRNA expression vector or the scrambled control and co-transfected with the (A) κB-Luc or (B) GRE-Luc plasmid were stimulated with TNF (10 ng/ml), Dex (0.5 mM) or Dex plus TNF and reporter activity was determined after 24 h. The diagram bars correspond to the average of relative light units (R.L.U.) normalized with the corresponding β-Galactosidase values. (C) Diagram bars correspond to the amplified scale of R.L.U. of κB-Luc and GRE-Luc activity in the presence of RAC3 siRNA. In all cases, results were analyzed by Tukey's Test.

Finally, RAC3 is an oncogene [27,40] that contributes to tumor development when is overexpressed. An inflammatory response, systemic or restricted to the tumor micro-environment plays an important role in oncogenesis and some inflammatory cytokines such as TNF, may contribute to tumorigenesis [28]. Therefore, the possible RAC3 up-regulation by the inflammatory response in tumors and surrounding tissues could be an important mechanism that deserves special attention and research for a better understanding of this disease.

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