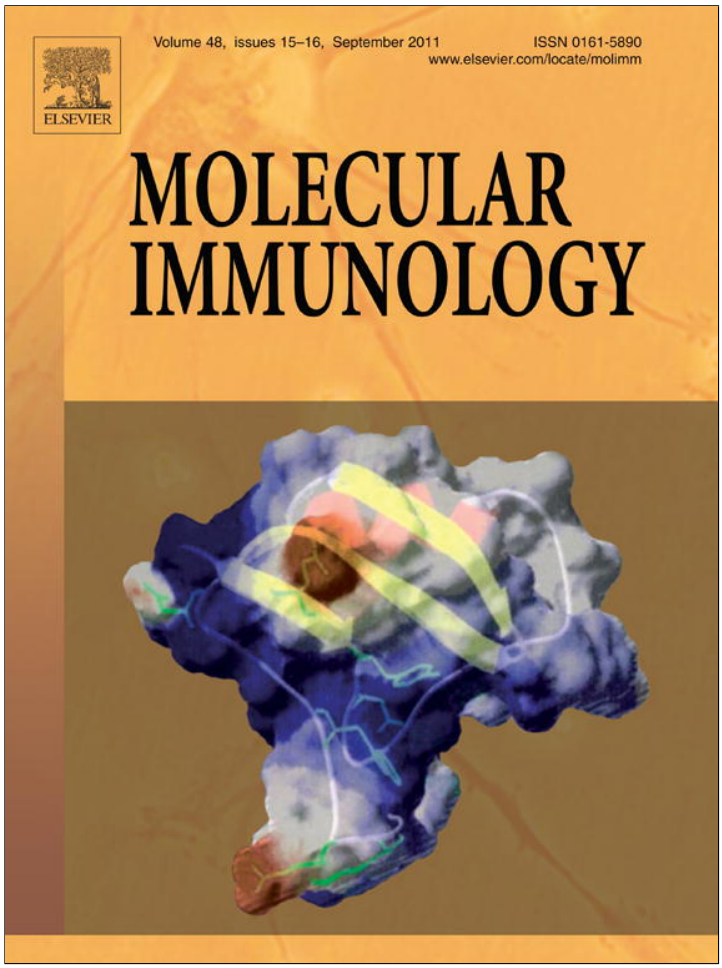

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Nuclear factor (NF)-κB controls expression of the immunoregulatory glycan-binding protein galectin-1

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ABSTRACT

The inflammatory response is a self-limiting process which involves the sequential activation of signaling pathways leading to the production of both pro- and anti-inflammatory mediators. Galectin-1 (Gal-1), an endogenous lectin found in peripheral lymphoid organs and inflammatory sites, elicits a broad spectrum of biological functions predominantly by acting as a potent anti-inflammatory factor and as a suppressive agent for T-cell responses. However, the molecular pathways underlying Gal-1 expression and function remain poorly understood. Here we identified a regulatory loop linking Gal-1 expression and function to NF-κB activation. NF-κB-activating stimuli increased Gal-1 expression on T cells, an effect which could be selectively prevented by inhibitors of NF-κB signaling. Accordingly, transient transfection of the p65 subunit of NF-κB was sufficient to induce high Gal-1 expression. Using *in silico* studies and chromatin immunoprecipitation analysis we have identified a functional NF-κB binding site within the first intron of the *LGALS1* gene. In addition, our results show that exogenous Gal-1 can attenuate NF-κB activation, as shown by inhibition of IκB-α degradation induced by pro-inflammatory stimuli, higher cytoplasmic retention of p65, lower NF-κB DNA binding activity and impaired transcriptional activation of target genes. The present study suggest a novel regulatory loop by which NF-κB induces expression of Gal-1, which in turn may lead to negative control of NF-κB signaling.

1. Introduction

During the past few years, there has been increasing interest in the impact of protein–glycan interactions in the regulation of innate and adaptive immune responses (van Kooyk and Rabinovich, 2008). Galectins are a family of soluble lectins defined by a common structural fold and a conserved carbohydrate recognition domain (CRD) of about 130 amino acids that recognizes glycans containing the disaccharide *N*-acetyl-lactosamine [Gal-β(1-4)-GlcNAc] (Rabinovich and Toscano, 2009). To date, as many as 15 galectins have been identified in mammals (Liu and Rabinovich, 2010). Some galectins (galectins 1, 2, 5, 7, 10, 11, 13, 14 and 15), which are traditionally classified as 'prototype' galectins, have one CRD that can dimerize, whereas others (galectins 4, 6, 8, 9 and 12), so called 'tandem-repeat' galectins, contain two homologous CRDs in tandem in a single polypeptide chain. Galectin-3 contains a CRD connected to a non-lectin N-terminal region that is responsible for oligomerization of this lectin (Nieminen et al., 2007; Liu and Rabinovich, 2010).

Galectin-1 (Gal-1), elicits a broad spectrum of biological functions predominantly by acting as a potent anti-inflammatory factor

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and as a suppressive agent for T-cell responses (Norling et al., 2008; Perone et al., 2006; Rabinovich et al., 1999; Santucci et al., 2003; Toscano et al., 2006, 2007). Particularly, administration of Gal-1 or its genetic delivery suppresses chronic inflammation in several models of autoimmunity by skewing the balance of the immune response toward a T helper (Th)2 cytokine profile (Rabinovich et al., 1999; Santucci et al., 2003; Toscano et al., 2006, 2007). Moreover, blockade of Gal-1 expression in tumor tissue results in heightened T cell-mediated tumor rejection and increased secretion of Th1-type cytokines (Rubinstein et al., 2004; Juszczynski et al., 2007). A potential mechanism underlying these immunosuppressive properties involves glycan-dependent control of T helper cell death. Specifically, we found that Th1 and Th17 effector cells express the repertoire of cell surface glycans that are essential for Gal-1 binding and subsequent cell death. By contrast, Th2 cells are resistant to Gal-1 binding through differential α2,6 sialylation of cell surface glycoproteins (Toscano et al., 2007). In addition, we have identified a hierarchy of tolerogenic signals triggered by Gal-1-glycan interactions and propagated by IL-27 and IL-10, which leads to differentiation of tolerogenic dendritic cells (DCs) capable of silencing pathogenic Th1 and Th17 responses (Ilarregui et al., 2009). Consistent with the anti-inflammatory role of this endogenous lectin, Gal-1-deficient (*Lgals1*$^{-/-}$) mice show greater Th1 and Th17 responses and are considerably more susceptible to immune-mediated fetal rejection and autoimmune disease than their wild-type counterparts (Blois et al., 2007; Toscano et al., 2007), suggesting an essential role for this glycan-binding protein in the control of immune tolerance and homeostasis.

Under certain inflammatory conditions, activated macrophages (Rabinovich et al., 1996, 1998), activated T lymphocytes (Blaser et al., 1998; Fuertes et al., 2004), activated B cells (Zuñiga et al., 2001) and alloreactive T cells (Rabinovich et al., 2002) secrete high amounts of Gal-1. Moreover, $CD4^+CD25^+FoxP3^+$ regulatory T (T_{reg}) cells synthesize substantial levels of Gal-1 which contributes to the immunosuppressive activity of these cells (Garin et al., 2007). Expression of Gal-1 appears to require TCR engagement and signaling pathways mediated by Lck and Fyn kinases (Fuertes et al., 2004). However, the mechanisms and molecular targets directly responsible for the regulated expression of Gal-1 in response to immune-activating and pro-inflammatory signals still remain uncertain.

The NF-κB transcription factors are central mediators of inducible transcription in the immune system and play essential roles in the initiation of innate and adaptive immune responses. Mammals have five NF-κB/Rel proteins, known as NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), RelA (also called p65), c-Rel and RelB, which can form homodimers or heterodimers (Ghosh and Hayden, 2008; Li et al., 2004; Pasparakis, 2009; Vallabhapurapu and Karin, 2009). These proteins are typically sequestered in the cytosol of unstimulated cells via interactions with specific inhibitory proteins known as IκB (Ghosh and Hayden, 2008; Li et al., 2004; Pasparakis, 2009). Activating stimuli trigger signaling cascades that cause the phosphorylation of IκB. As a result, the ubiquitin ligase complex mediates polyubiquitination of IκB, and leads to its subsequent proteasomal degradation. NF-κB can be further activated by post-translational modifications and translocate to the nucleus where it binds to specific NF-κB DNA sequences in order to regulate expression of target genes (Ghosh and Hayden, 2008; Li et al., 2004; Pasparakis, 2009).

Activating stimuli such as pro-inflammatory cytokines (TNF, IL-1), microbial components or antigen receptors mainly activate the canonical NF-κB signaling pathway, which involves the activation of IκB kinase (IKK) complex, IκB-α degradation and dissociation from RelA:p50 or c-Rel:p50 dimers. On the other hand, the non-canonical (alternative) NF-κB pathway is activated by receptors that mediate lymphoid tissue organogenesis and lymphocyte development, such as the lymphotoxin-β receptor which induce processing of p100 to p52 and nuclear accumulation of p52–RelB dimers (Ghosh and Hayden, 2008; Pasparakis, 2009).

Prompt activation of NF-κB is required to mount a successful immune response; yet this response needs to be properly terminated to avoid tissue damage and uncontrolled inflammation which may increase the risk of autoimmune disease (Ghosh and Hayden, 2008; Vallabhapurapu and Karin, 2009). Interestingly, Gal-1 expression is up-regulated during the peak and recovery phases of inflammatory responses (Ilarregui et al., 2009), suggesting a critical role of this endogenous lectin in promoting resolution of inflammation and a possible functional link between this transcription factor and Gal-1 expression. In order to address this hypothesis, we examined the potential role of the NF-κB signaling pathway in regulating expression and function of this endogenous lectin. Here, we provide evidence of a novel regulatory loop where NF-κB controls expression of Gal-1, which in turn leads to attenuation of NF-κB signaling. Our findings suggest that NF-κB-dependent Gal-1 expression on effector T cells may play a role in limiting inflammatory responses and tempering autoimmune processes.

2. Materials and methods

2.1. Reagents

Ficoll-Paque Plus was obtained from Amersham Biosciences (Piscataway, NJ). RPMI 1640, Fetal Calf Serum (FCS), L-glutamine and gentamicin were obtained from Gibco Life Technologies (Carlsbad, CA). Phytohemagglutinin, human TNF, human IL-4, sulfasalazine, Ly294002 and U0126 were purchased from Sigma (St. Louis, MO). Human IL-12 was purchased from BD Biosciences (Mountain View, CA). SB202190 and FK506 were obtained from Calbiochem (Darmstadt, Germany). Anti-IL-4 (clone 8D4-8), anti-IL-12 (clone C8.6), and anti-CD3ε (clone SK7) were purchased from BD Biosciences (San Diego, CA). Recombinant human Gal-1 (rhGal-1) was produced and purified as described (Barrionuevo et al., 2007) and rabbit policlonal anti-Gal-1 antibody was obtained as previously described (Rabinovich et al., 1999; Rubinstein et al., 2004). Antibodies against IκB-α, RelA/p65, p50 and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Peroxidase labeled anti-rabbit IgG was obtained from Bio Rad (Hercules, CA).

2.2. Isolation of human peripheral blood mononuclear cells and culture conditions

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy human volunteers by Ficoll-Paque Plus gradient centrifugation, washed, and resuspended in RPMI 1640 supplemented with 10% (v/v) heat-inactivated FCS, 2 mM L-glutamine and 100 μg/ml gentamicin. When indicated, monocytes were removed by plastic adherence, incubating for 2 h at 37 °C in Petri dishes. Cells were cultured at 1×10^6 cells/ml and kept at 37 °C and 5% CO_2 during the duration of experiments.

For polarization, cells were separated into three groups: activated (Act), Th1 and Th2 cells. These cells were cultured with 1 μg/ml phytohemagglutinin (PHA-L) alone or under polarizing conditions (Th1: 2 ng/ml IL-12, 100 ng/ml anti-IL-4; Th2: 5 ng/ml IL-4, 2 μg/ml anti-IL-12) for 5 days. Pharmacological inhibitors or control diluents (DMSO or ethanol) were included for the last 24 h of culture. The concentrations used were 10 and 30 μM of U0126, 1 and 5 μM of FK506, 10 and 30 of μM SB2021902, 2 and 4 μM of Ly294002 and 0.5 and 2 mM of sulfasalazine. Controls of the efficacy of inhibitors were performed as described (Ilarregui et al., 2009). In another set of experiments PBMCs were incubated with anti-CD3

mAb (25 ng/ml) or TNF (10 ng/ml) and sulfasalazine or DMSO was added during the entire culture period.

All protocols were approved by the Institutional Review Board of the Institute of Biology and Experimental Medicine.

2.3. SDS-PAGE and Western blot analysis

Cells were washed with cold phosphate buffered saline (PBS) and total cell lysates were prepared with lysis buffer [150 mM NaCl, 50 mM Tris–HCl, pH 7.5, 10 mM EDTA, 1% (v/v) Nonidet P-40] in the presence of a cocktail of protease inhibitors (1:100; Sigma). After 1 h incubation on ice, lysates were centrifuged for 20 min at 10,000 rpm at 4 °C. Supernatants were collected and stored at −20 °C. To analyze secreted proteins, conditioned media were obtained by washing cells and culturing them in RPMI 1640, 2 mM glutamine, 100 μg/ml gentamicin without FCS for 18 h. After centrifugation at 2000 rpm for 10 min, cells were discarded and proteins were precipitated with 4 vol of acetone at −20 °C for 6 h. Then, precipitated proteins were centrifuged at 3000 rpm for 30 min at 4 °C, the supernatant was removed and the pellet dried. Finally, pellets were dissolved in PBS 0.5× with protease inhibitors and centrifuged at 10,000 rpm for 5 min at 4 °C. To obtain cytoplasmic extracts, cells were washed with cold PBS and resuspended in hypotonic buffer (10 mM Tris–HCl, 0.2 mM $MgCl_2$, pH 6.7) with protease inhibitors. Cells were incubated 5 min on ice and centrifuged for 10 min at 1500 rpm at 4 °C. Protein concentration was estimated using the MicroBCA kit (Pierce).

SDS-PAGE and Western blot analysis were performed as previously described (Rubinstein et al., 2004). Equal amounts of protein of cell lysates and supernatants were resolved on a 15% SDS-PAGE. Separated proteins were electroblotted onto nitrocellulose membranes (Amersham Biosciences) and probed with anti-Gal-1 (1:5000 dilution), anti-IκB-α (1:1000 dilution), anti-p65 (1:1000 dilution) or anti-actin (1:3000 dilution) rabbit polyclonal antibodies. Membranes were then incubated with a peroxidase-labeled anti-rabbit IgG and developed using an enhanced chemoluminescence detection kit (Amersham) and Kodak BioMax films (Rochester, NY). Films were analyzed with the Scion Image Analysis software (Scion, Frederick, MD), and the intensity of each band was recorded and expressed as arbitrary units (AU).

2.4. Transfections

The expression vector CMV-Rel-A encoding human p65(RelA) was kindly provided by J. DiDonato (Lerner Research Institute, Cleveland Clinic, Cleveland, OH). κB-Luc reporter plasmid was kindly provided by O. Coso (Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina). Transient transfection experiments in HEK293 cells were performed using Lipofectamine 2000 (Invitrogen), following the instructions provided by the manufacturer. CMV-Rel-A plus RSV-β-gal transfected cells were cultured for 48 h, harvested, and used for analysis of Gal-1 expression by Western blot. Mock-transfected cells were also analyzed. κB-Luc-transfected cells were incubated for 18 h in the presence or absence of rhGal-1 and/or TNF. Cellular extracts were prepared and Luciferase and β-gal assays were performed as described (Werbajh et al., 2000).

2.5. Electrophoretic mobility shift assays

Electrophoretic mobility shift assays (EMSA) were performed as previously described (Costas et al., 1996). Nuclear extracts were obtained from PBMCs stimulated for 1 h with TNF (10 ng/ml) in the absence or presence of rhGal-1. Cells were lysed with 10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and 0.1% (v/v) Nonidet P-40. After centrifugation, nuclear pellets were extracted with 20 mM HEPES, 1.5 mM $MgCl_2$, 0.42 mM NaCl, 0.5 mM DTT, 0.5 mM PMSF, 25% (v/v) glycerol and 0.2 mM EDTA. The soluble fraction was mixed with 10 mM Tris–HCl, 80 mM KCl, 10% (v/v) glycerol and 1 mM DTT, pH 7.5. Double-stranded oligonucleotides containing the NF-κB consensus binding site located in the enhancer of the Ig_κ chain gene (AGTTGAGGGGACTTTCCCAGGC, κB-cons; Santa Cruz Biotechnology) were labeled with ^{32}P-ATP and used for binding reactions. Five micrograms of each nuclear extract were incubated in 20 μl of buffer containing 100 ng of poly(dI-dC). After incubation for 20 min at room temperature with labeled oligonucleotides, DNA–protein complexes were resolved on a 6% nondenaturing polyacrylamide gel with 0.25× TBE buffer. Gels were dried and autoradiography was performed at −70 °C. For competition experiments, a 100- or 200-fold excess of unlabeled κB-cons oligonucleotide was added to the reaction mixture.

2.6. Analysis of transcription factor binding sites

The gene encoding human galectin-1 (*LGALS1*; GeneID: 3956) is located in chromosome 22 between 38,071,613 to 38,075,809 bp (www.ncbi.nlm.nih.gov/gene/3956). Analysis for detection of putative regulatory sequences present in the *LGALS1* gene (within a region encompassing approximately 2500 bp up- and down-stream from the transcription start site) was performed using the publicly available version of MatInspector software (www.genomatix.de) (Cartharius et al., 2005).

2.7. Chromatin immunoprecipitation

PBMCs were incubated with PHA-L (1 μg/ml) for 24 h. Protein–DNA cross-linking was performed by incubating the cells with 1% (v/v) formaldehyde for 10 min at 37 °C, after which glycine (0.125 M) was added to quench the reaction. Cells were lysed in RIPA buffer [150 mM NaCl, 1% (v/v) Nonidet-P40, 0.5% (w/v) deoxycolate, 0.1% (w/v) SDS, 50 mM Tris, pH 8, 5 mM EDTA] with protease inhibitor cocktail (Sigma). The lysate was sonicated to obtain chromatin fragments of an average size of 300–500 bp. Cellular debris was removed by centrifugation at 12,000 rpm for 10 min at 4 °C. Supernatants were precleared by incubation for 2 h at 4 °C with protein A/protein G-Sepharose (15 μl for each mg protein; Santa Cruz Biotechnology). An aliquot of pre-cleared chromatin was taken (input) and used in the subsequent PCR analysis since it contained all possible DNA fragments in genomic proportions. The remaining chromatin was incubated with 3 μg of mouse anti-p50 (sc-8414; Santa Cruz Biotechnology), rabbit anti-acetyl histone H3 (07-353, Upstate Biotechnology, Lake Placid, NY), normal mouse IgG (sc-2025; Santa Cruz Biotechnology) or normal rabbit IgG (sc-2027, Santa Cruz Biotechnology). Immune complexes were purified by treatment with protein A/protein G-Sepharose, followed by washing with RIPA and IP buffers [IP buffer: 100 mM Tris–HCl pH 8.5, 500 mM LiCl, 1% (v/v) Nonidet P-40, 1% (w/v) deoxicolate]. Cross-links were reversed by incubation with 190 mM Tris–HCl pH 6.8, 15% (v/v) 2-mercaptoethanol and 6% (w/v) SDS for 40 min at 100 °C. DNA was purified by phenol/chloroform extraction followed by ethanol precipitation.

2.8. PCR analysis

Relative template enrichment after immunoprecipitation with respect to the input was determined by real time PCR in a MJ research Opticon system (Waltham, MA) using the Opticon Monitor 3.1 software. Primers for detection of NF-κB-binding site G1-7 (forward: TTGAGTCCAAAATTCCCAAG; reverse: TGTTCCCGCCCGGCATCCTC) and for detection of the negative control sequence G1-N (forward: GTGGCATGGCCAGAGCTAGA; reverse: GAAGTGCAGGCACAGGTTGT) were designed to yield fragments of

approximately 200 bp in both cases. Amounts of template for each immunoprecipitated sample or input were obtained in quadruplicate and expressed as arbitrary units from calibration curves. The specific signal was calculated as the difference between the mean value obtained from specific antibodies and that of their respective isotype control and subsequently normalized by input values. Results were expressed as fold increase relative to the input value.

2.9. Confocal microscopy

PBMCs were fixed in 4% (w/v) paraformaldehyde for 30 min, permeabilized and blocked in 10% (v/v) FCS, 0.05% (v/v) Triton X-100 in PBS and incubated with anti-p65 polyclonal Ab (1:100 dilution) for 2 h on ice. Then cells were washed, incubated for 1 h with FITC-labeled anti-rabbit IgG (1:100 dilution) and mounted with Vectashield (Vector Labs). Nuclei were stained with propidium iodide and negative controls lacked primary antibodies. Cells were analyzed on a Nikon laser confocal microscope (Eclipse E800).

2.10. Statistical analysis

Comparison of two groups was made using the Student's *t*-test for unpaired data when appropriate using Prism software (GraphPad). *P*-Values of 0.05 or less were considered statistically significant.

3. Results

3.1. Activated and differentiated cells express high amounts of Gal-1

To understand the mechanisms involved in Gal-1 expression and function, we first analyzed the pattern of expression and secretion of Gal-1 in human peripheral blood mononuclear cells (PBMCs) activated with PHA-L and under neutral, Th1 or Th2 polarizing conditions. Western blot analysis of whole cell lysates and conditioned media showed that activated, Th1 and Th2 cells synthesized and secreted abundant levels of Gal-1 after 5 days of stimulation (Fig. 1A). Comparison of the expression pattern of Gal-1 on Th1 and Th2 cells in a time course study (spanning a 5-day period subsequent to activation) revealed that both cells types increase Gal-1 expression at similar rates, reaching a maximum on day 5 (Fig. 1B). Analysis of conditioned media during the course of the culture period revealed that human Th1 cells secrete higher amounts of Gal-1 than human Th2 cells (Fig. 1C). These results suggest that expression of Gal-1 is induced during T helper cell differentiation and that secretion of this lectin is differentially regulated in Th1 and Th2 cells.

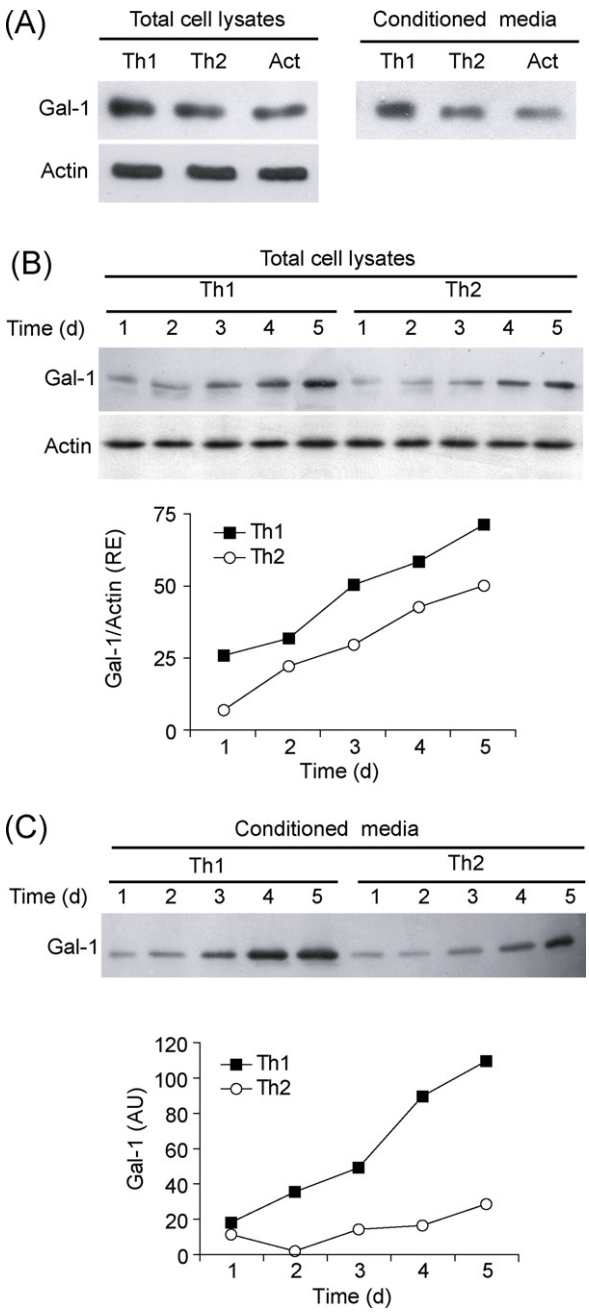

Fig. 1. Time-course analysis of the regulation of Gal-1 expression and secretion during T cell activation and T helper cell differentiation. PBMCs were depleted of monocytes and activated under neutral conditions (Act) or differentiated toward Th1 or Th2 profiles during 5 days. (A) Detection of Gal-1 expression (total cell lysates) and secretion (conditioned media) by Western blot. (B) Kinetics of Gal-1 expression during the course of Th1 and Th2 differentiation. Relative expression (RE): band intensity relative to that of actin. (C) Kinetics of Gal-1 secretion during Th1 and Th2 differentiation. AU: arbitrary units. Results are representative of three independent experiments.

3.2. Signaling pathways involved in Gal-1 expression

To examine the intracellular signals involved in the control of Gal-1 expression on Th1, Th2 and activated non-polarized T cells, we performed a screening study using pharmacological inhibitors of the p38 (SB202190), PI3K/AKT (Ly294002), ERK1/2 (U0126), calcineurin/NFAT (FK506) and NF-κB (sulfasalazine) signaling pathways. Cells were polarized for 5 days and further incubated with increasing concentrations of the inhibitors for the last 24 h. Addition of inhibitors at the end of the differentiation period, allowed us to study Gal-1 expression in fully activated and differentiated cells. We could not observe a clear modulation of Gal-1 expression on T cells differentiated under Th1, Th2 or neutral conditions when inhibitors of p38 (Fig. 2A), PI3K/AKT (Fig. 2B), ERK1/2 (Fig. 2C), or calcineurin/NFAT (Fig. 2D) were added to cell cultures at increasing concentrations. However, inhibition of NF-κB signaling resulted in marked downregulation of Gal-1 expression (Fig. 2E). This effect was more evident on Th1 cells compared to Th2 or non-polarized T cells. These results suggest that the NF-κB signaling pathway is involved in Gal-1 synthesis during T cell activation and differentiation.

3.3. The NF-κB signaling pathway is involved in controlling Gal-1 expression

To further investigate the involvement of NF-κB in the induction of Gal-1 expression, cells were exposed to potent activators

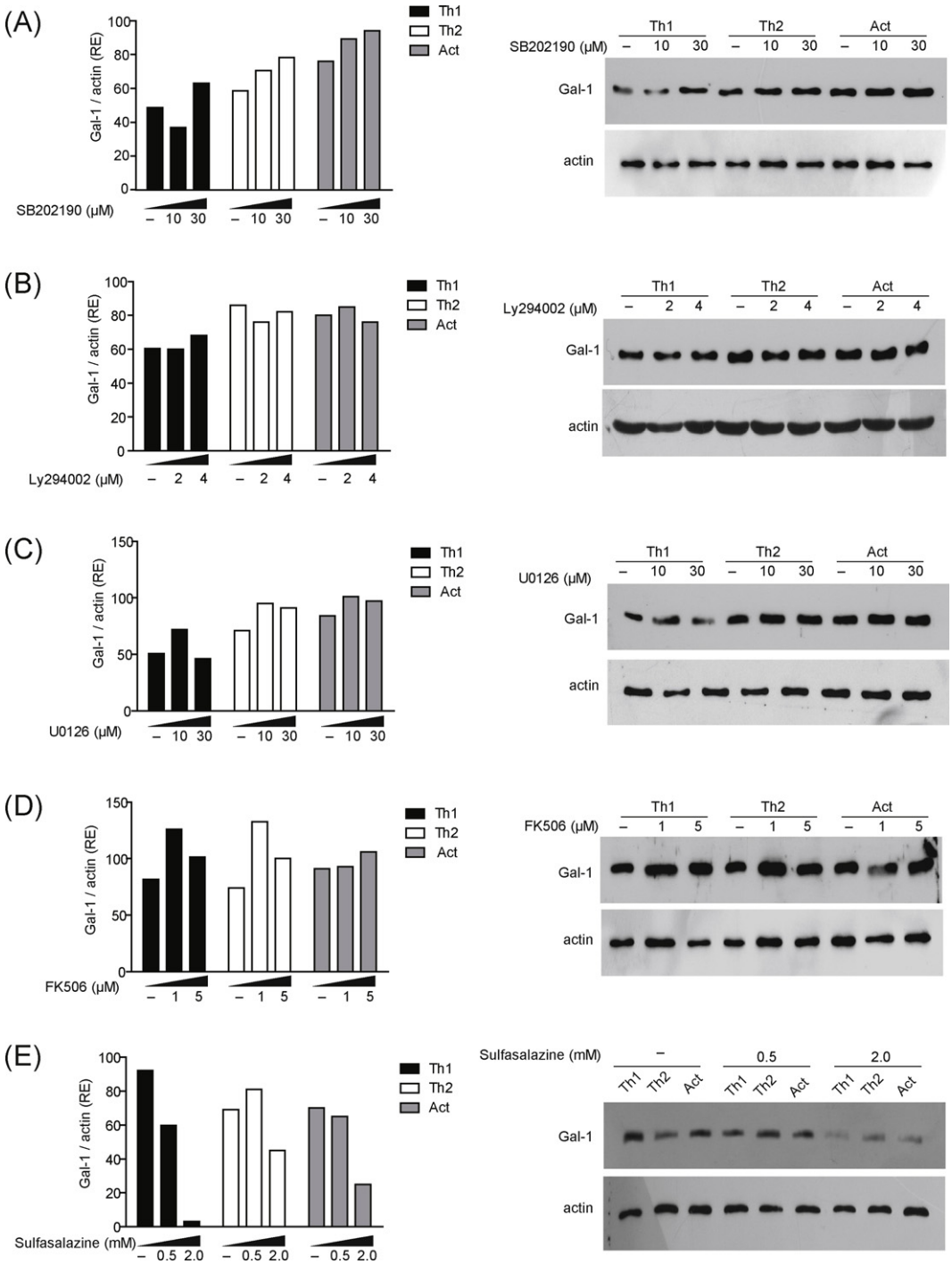

Fig. 2. Signaling pathways involved in Gal-1 expression in fully activated and differentiated T cells. PBMCs were depleted of monocytes and activated (Act) or differentiated into Th1 or Th2 profiles during 5 days and incubated with different pharmacological inhibitors during the last 24 h. Expression of Gal-1 on cells incubated in the presence of inhibitors of p38 (SB202190; 10 and 30 μM) (A), PI3/AKT (Ly294002; 2 and 4 μM) (B), ERK1/2 (U0126; 10 and 30 μM) (C), calcineurin/NFAT (FK506; 1 and 5 μM) (D) and NF-κB (sulfasalazine; 0.5 and 2 mM) (E). RE: band intensity relative to that of actin. Results are representative of three independent experiments.

of NF-κB signaling (i.e., engagement of the TCR/CD3 complex and addition of the pro-inflammatory cytokine TNF) in the absence or presence of sulfasalazine. Similarly to the effects of PHA-L, TCR cross-linking using an anti-CD3 antibody induced Gal-1 expression which could be prevented by sulfasalazine in a dose-dependent manner (Fig. 3A). Moreover, incubation of cells with TNF for 18 h increased Gal-1 synthesis, an effect which could be inhibited by sulfasalazine (Fig. 3B).

Stimulation through CD3/TCR complex or TNF receptors can trigger a broad spectrum of signaling cascades independent of NF-κB activation (Costas et al., 1996; Fuertes et al., 2004). In order to narrow down the number of possible effectors involved in Gal-1 induction and to extend the putative role of NF-κB to other non-immune target cells, we transiently expressed the NF-κB p65 subunit in HEK293 cells and analyzed Gal-1 expression after 48 h. Remarkably, transient transfection of p65 was sufficient to markedly increase Gal-1 expression (Fig. 3C). These results indicate that Gal-1 expression is regulated by NF-κB activating stimuli independently of the T cell activation process, an effect which was further substantiated by overexpression of the p65 subunit.

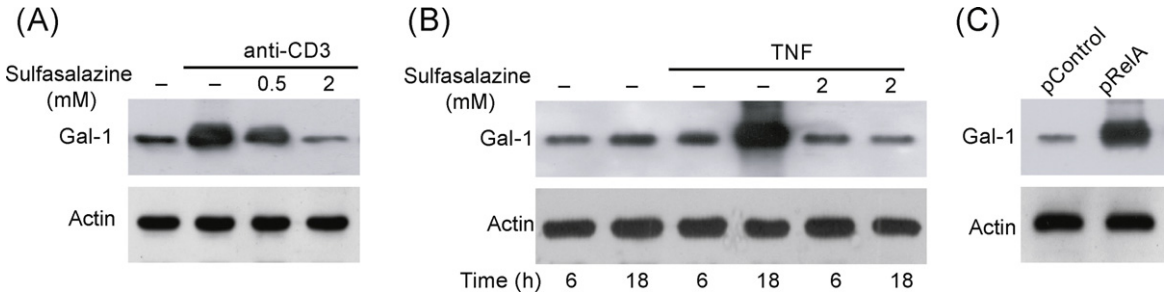

Fig. 3. The NF-κB signaling pathway regulates Gal-1 expression. (A) Western blot analysis of Gal-1 expression in monocyte-depleted PBMCs stimulated with anti-CD3 mAb (25 ng/ml) in the absence or presence of sulfasalazine (0.5 and 2 mM) for 3 days. (B) Western blot analysis of Gal-1 expression in monocyte-depleted PBMCs cultured in the presence or absence of TNF (10 ng/ml) and/or sulfasalazine (2 mM) during 6 and 18 h. (C) Western blot analysis of Gal-1 expression in HEK293 cells transiently transfected with a plasmid encoding the p65 subunit or empty vector as control. Results are representative of three or four independent experiments.

3.4. *LGALS1* gene is a direct target of NF-κB

In order to evaluate whether NF-κB can directly regulate Gal-1 expression by interacting with regulatory sequences in the *LGALS1* gene, we performed an *in silico* analysis aimed to detect putative binding sites for this transcription factor using MatInspector software (www.genomatix.de) (Cartharius et al., 2005). Sequence analysis encompassing approximately 5000 bp centered around the transcription start site of the *LGALS1* gene revealed several putative NF-κB binding sites with core similarity of 1 and matrix similarities above 0.9 (Table 1).

Given that prediction programs can infer transcription factor binding sites, yet they do not estimate the functionality of these sites, we performed chromatin immunoprecipitation assays using PCR primers designed to amplify DNA fragments harboring putative NF-κB binding sequences and a control fragment (G1-N) lacking consensus sequences for NF-κB (Fig. 4A). Immunoprecipitation of DNA samples from PHA-L-activated PBMCs with an anti-p50 antibody and subsequent quantitative PCR analysis revealed significant binding of the p50 subunit to one of the predicted NF-κB binding sites downstream the *LGALS1* transcriptional starting point (G1-7) as compared to the control G1-N sequence (Fig. 4B). Moreover, using an anti-acetyl-histone H3 antibody, we found evidence for chromatin remodeling nearby the G1-7 NF-κB binding sequence (Fig. 4C). This finding indicates that G1-7 may act as a regulatory ele-

Table 1
Putative NF-κB-binding sites present in the *LGALS1* gene.

#	Position	Strand	Core	Matrix	Sequence
1	−2378	+	1	0.932	ttgGGGAggcccg
2	−511	+	1	0.901	cgGGGAaggcccg
3	74	–	1	0.894	ggaggatgTTCCc
4	113	+	1	0.934	gtgGGGAcccccc
5	409	+	1	0.917	tggGGGAcaccca
6	603	+	1	0.946	gggggaaaTTCCc
7	1185	+	1	0.995	taGGGActttccc

Analysis of putative transcription factor binding sites was performed using the MatInspector Software. Sites with matrix similarity values above 0.9 found in a region ranging from approximately 2500 bp upstream to 2500 bp downstream of the transcription start site of *LGALS1* gene are shown. The core binding site for NF-κB is denoted in capitals.

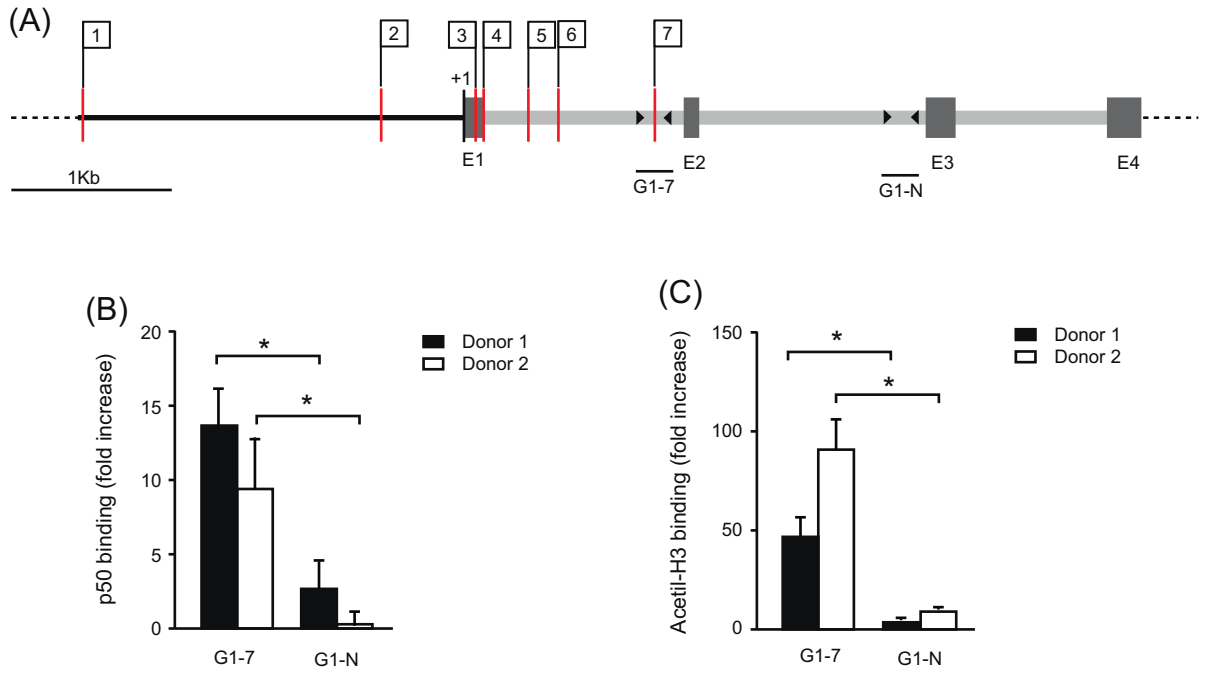

Fig. 4. NF-κB/p50 binds to regulatory elements in the *LGALS1* gene. (A) Schematic representation of seven NF-κB binding sites found in the *LGALS1* gene (numbered flags). Analysis of sequences ranging from approximately 2500 bp upstream to 2500 downstream of the transcription starting site was performed using MatInspector software. G1-7, fragment containing the NF-κB binding site number 7. G1-N, fragment with no NF-κB binding sequences. (B and C) Chromatin immunoprecipitation and PCR analysis of G1-7 and G1-N fragments with anti-p50 (B) and anti-acetyl-H3 histone (C) Abs. Total PBMCs from two independent donors (1 and 2) were activated with PHA-L (1 μg/ml) for 24 h. Results are expressed as fold increased respect to input. $^{*}P < 0.01$.

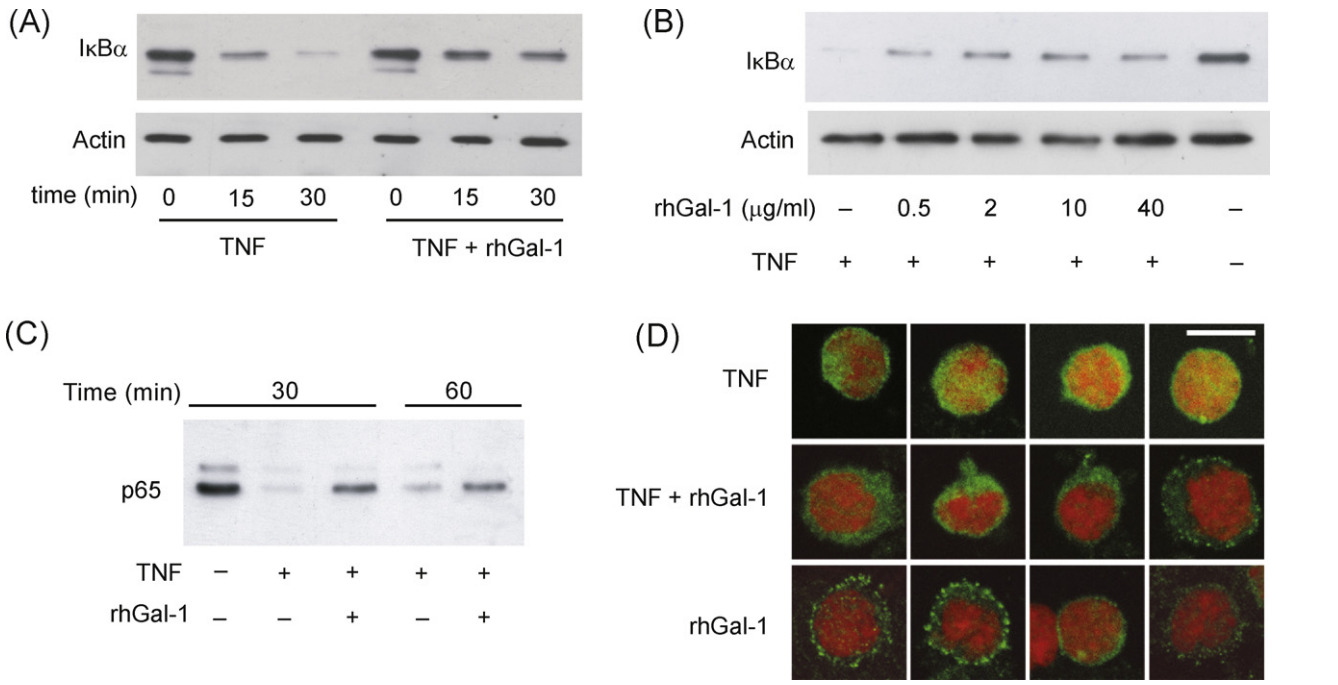

Fig. 5. Gal-1 inhibits IκB-α degradation and nuclear translocation of NF-κB. (A) Western blot analysis of IκB-α in whole cell extracts from total PBMCs incubated with TNF (10 ng/ml) in the presence or absence of rhGal-1 (10 μg/ml) for 15 and 30 min. (B) Western blot analysis of IκB-α in total PBMCs incubated with TNF (10 ng/ml) in the absence or presence of increasing doses of rhGal-1 (0.5, 2, 10 and 40 μg/ml) for 30 min. (C) Western blot analysis of cytoplasmic p65 in total PBMCs incubated with TNF (10 ng/ml) in the absence or presence of rhGal-1 (10 μg/ml) for 30 and 60 min. (D) Laser confocal microscopy of p65 (green) in total PBMCs incubated with TNF (20 ng/ml) in the absence or presence of rhGal-1 (20 μg/ml) for 45 min. Nuclei were counterstained with propidium iodide (red). Scale bar, 10 μm. Photographs are representative of three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ment since histone acetylation drives transcription by favoring an open chromatin conformation that allows binding of transcription factors (Imhof and Wolffe, 1998). These results indicate that NF-κB p50 subunit directly interacts with transcriptional active regions in the *LGALS1* gene.

3.5. Reciprocal control of NF-κB signaling by exogenous Gal-1

The NF-κB pathway has been typically implicated in the initiation and amplification of innate and adaptive immune responses through activation of pro-inflammatory transcriptional programs (Pasparakis, 2009). Yet, our results indicate that expression of Gal-1, an endogenous lectin with anti-inflammatory and immunoregulatory properties, is also regulated by NF-κB. Hence, we hypothesized that Gal-1 might represent a novel inhibitory mediator of the NF-κB signaling pathway. To test this hypothesis, we stimulated PBMCs with TNF in the absence or presence of rhGal-1 for short time periods. We found that rhGal1 inhibits IκB-α degradation induced by TNF (Fig. 5A) at 15 and 30 min post-stimulation. This inhibitory effect was evident at low concentrations of rhGal-1 and reached a peak with a concentration of 10 μg/ml (Fig. 5B). Thus, exogenous Gal-1 can effectively prevent TNF-induced IκB-α degradation.

To further dissect the molecular events involved in the NF-κB signaling pathway, we analyzed the translocation of NF-κB to the nucleus using subcellular fractioning. Incubation of cells with rhGal-1 resulted in higher cytoplasmic retention of p65 reflecting a diminished translocation of p65 to the nucleus (Fig. 5C and D). This effect was confirmed by confocal microscopy showing that cells incubated with TNF plus rhGal-1 had decreased nuclear p65 levels as compared to cells stimulated with TNF alone (Fig. 5D). These results were also confirmed by analyzing the availability of nuclear NF-κB to bind to its consensus sequences using EMSA and a specific ELISA for this transcription factor in PBMCs incubated with TNF in the absence or presence of rhGal-1. We observed lower DNA binding activity of NF-κB (Fig. 6A) and significantly reduced p50 binding to DNA consensus sequences (Fig. 6B) when cells were exposed to rhGal1 as compared to those incubated with TNF alone. To analyze the effect of rhGal-1 on the transcriptional response mediated by NF-κB, we transfected HEK293 cells with a reporter plasmid (κB-Luc) encoding Luciferase downstream of NF-κB-binding sites and incubated these cells with TNF in the absence or presence of rhGal-1. Remarkably, TNF-induced transcriptional activity of NF-κB was significantly prevented by addition of rhGal-1 (Fig. 6C). Incubation with rhGal-1 alone did not induce any change in Luciferase activity in the absence of other stimuli (Fig. 6C). These observations support a role of Gal-1 as an inhibitor of the NF-κB signaling pathway. Collectively, our findings suggest the existence of a novel regulatory loop involving an NF-κB/Gal-1 axis (Fig. 7); in response to pro-inflammatory or activation stimuli, NF-κB induces Gal-1 expression, which in turn may lead to inhibition of the NF-κB signaling pathway.

4. Discussion

The inflammatory response is characterized by the coordinated activation of various signaling pathways that regulate expression of both pro- and anti-inflammatory mediators (Vallabhapurapu and Karin, 2009). Compelling evidence indicates that the NF-κB pathway plays a central role in triggering inflammation and activation of adaptive immune responses. This knowledge is based on evidence showing that pro-inflammatory cytokines, including TNF and IL-1, as well as TCR engagement or TLR signaling trigger NF-κB activation (Li et al., 2004). These data is substantiated by the identification of pro-inflammatory cytokines, chemokines and cell adhesion molecules as target genes of NF-κB (Pasparakis, 2009). However, emerging data supports a regulatory function of this transcriptional pathway during the resolution of the inflammatory response and induction of immune cell homeostasis (Lawrence,

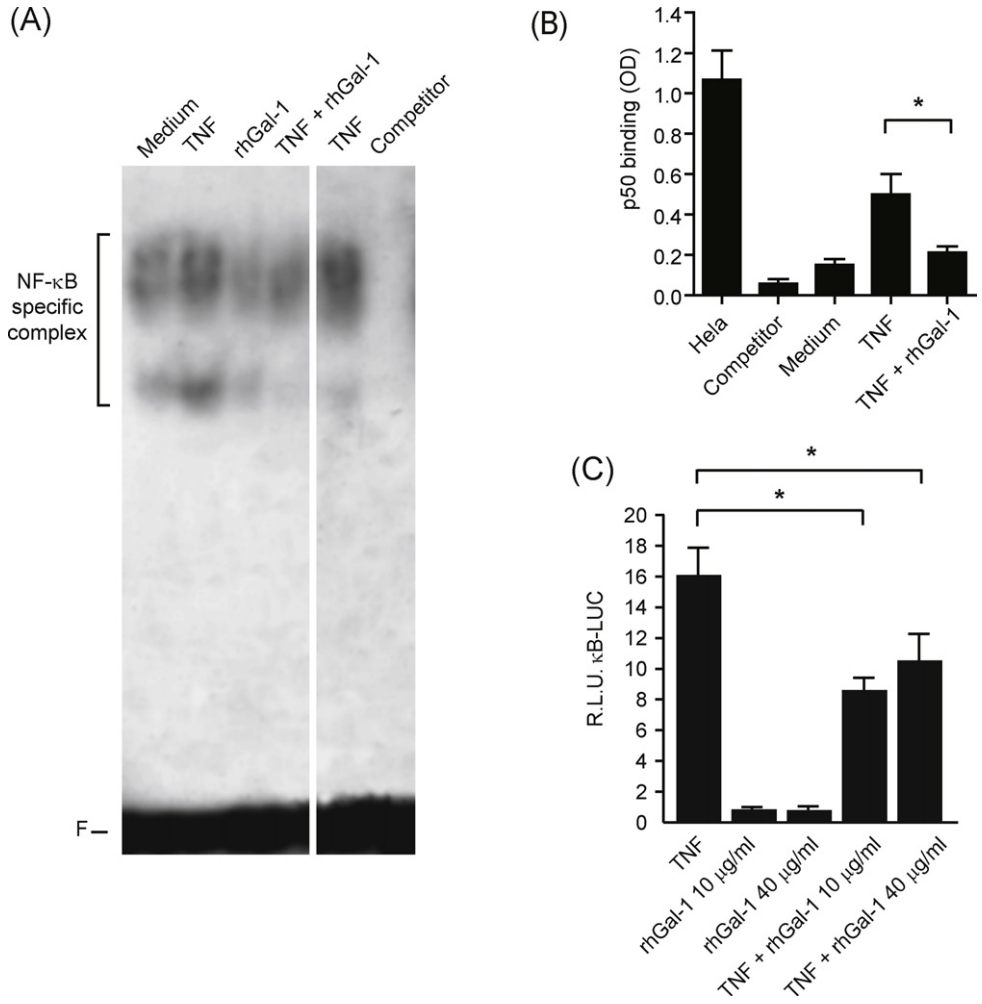

Fig. 6. Gal-1 attenuates NF-κB DNA binding and transcriptional activities. (A) EMSA of nuclear extracts from total PBMCs incubated with TNF (10 ng/ml) in the absence or presence of rhGal-1 (10 μg/ml) for 1 h. (B) ELISA for detection of the p50 subunit of NF-κB in nuclear extracts from total PBMCs incubated with TNF (10 ng/ml) in the absence or presence of rhGal-1 (10 μg/ml) for 1 h. $^*P < 0.05$. (C) The diagram bars shows the average ± SD of the relative light units (R.L.U.) respect to the constitutive β-galactosidase values of extracts from HEK293 cells transfected with a reporter plasmid (κB-Luc) encoding Luciferase downstream of NF-κB binding sites plus RSV-β-gal and incubated with TNF in the presence or absence of rhGal-1 (10 and 40 μg/ml) for 18 h. $^*P < 0.05$.

2009). The present study identifies the NF-κB pathway as a key regulator of the expression of Gal-1, an endogenous glycan-binding protein with anti-inflammatory activity (Barrionuevo et al., 2007; Ilarregui et al., 2009; Norling et al., 2008; Toscano et al., 2007).

Activation-induced cell death (AICD) is one of the major mechanisms involved in peripheral tolerance that ensures the elimination of activated lymphocytes after the completion of immune responses. The Fas/Fas ligand (FasL) system plays an essential role in this process by triggering AICD. FasL expression is kept under tight regulation being induced by T cell activating stimuli (Green et al., 2003). Notably, the Gal-1/glycan axis has striking similarities with the Fas/FasL system as it is also expressed on activated T cells and is capable of inducing apoptosis of activated and highly differentiated T helper cells (Perillo et al., 1995; Toscano et al., 2007). In addition, both the Gal-1/glycan axis, as well as the Fas/Fas L system appear to be regulated by NF-κB activation (Kavurma and Khachigian, 2003; Lin et al., 1999). Hence, although numerous studies support the critical role of NF-κB during the development of inflammatory responses, it is evident that this transcription factor is also involved in immune homeostatic processes, like AICD and the resolution of inflammatory responses, through induction of pro-apoptotic factors such as Gal-1 and FasL.

Inducible transcription factors remain activated only for defined periods of time, a feature that requires the proper function of delayed negative feedback loops (Renner and Schmitz, 2009). Here we found that NF-κB is responsible for inducing expression of Gal-1 which in turn attenuates the NF-κB signaling pathway. In this regard, Satelli and Rao, recently reported that intracellular Gal-1 inhibits key elements of the NF-κB signaling pathway such as phosphorylation of IKKα/β and p65 in the LS-180 colorectal carcinoma cell line (Satelli and Rao, 2011). Our results show that NF-κB-dependent Gal-1 expression may contribute to attenuate immune responses, at least in part, by limiting transcription of NF-κB-regulated pro-inflammatory target genes. In a similar scenario, the intracellular ubiquitin-editing protein A20 / TNFAIP3 is induced by NF-κB activating stimuli and is a key player in the negative regulation of NF-κB signaling (Coornaert et al., 2009; Vereecke et al., 2009). Notably, mice lacking A20 die shortly after birth due to multi-organ inflammation suggesting a key role for A20 in immune cell homeostasis (Coornaert et al., 2009; Vereecke et al., 2009). In this regard, Gal-1 has been intensively studied as an endogenous immunosuppressive factor and Gal-1-deficient mice show increased susceptibility to autoimmune neuroinflammation (Rabinovich and Toscano, 2009). The mechanisms underlying the anti-inflammatory activity of Gal-1 include the control of effector T cell survival (Motran et al., 2008; Stillman et al., 2006; Toscano et al., 2007), cytokine balance (Juszczynski et al., 2007; Rabinovich et al., 1999; Rubinstein et al., 2004; Stowell et al., 2008; van der

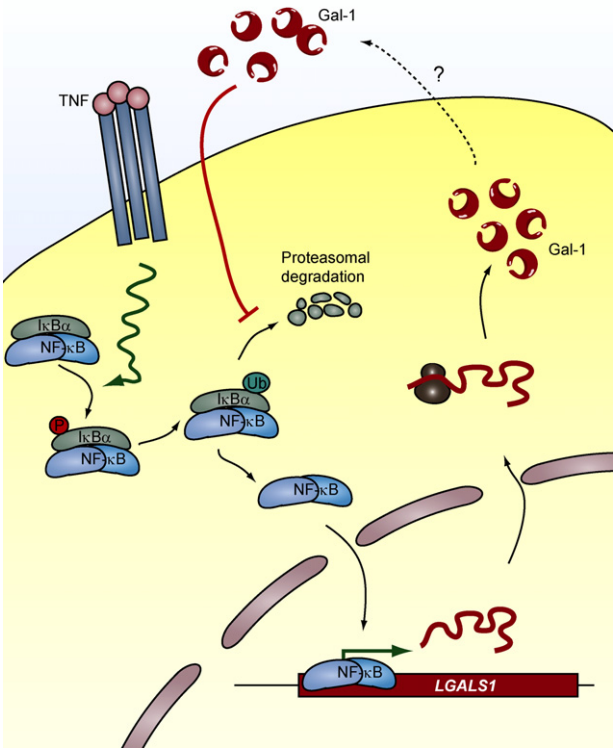

Fig. 7. Hypothetical model. Results presented in this work suggest the occurrence of an NF-κB-Gal-1-dependent regulatory loop. NF-κB activation triggered by inflammatory or activation stimuli induces Gal-1 expression, which in turn may suppress inflammatory responses by inhibiting NF-κB signaling.

Leij et al., 2007), T cell trafficking and migration (Norling et al., 2008), and generation of tolerogenic dendritic cells (Ilarregui et al., 2009). Given the relevance of NF-κB during the development of inflammatory processes (including survival, cytokine production, trafficking and dendritic cell physiology), Gal-1-induced inhibition of NF-κB signaling could be considered as an alternative explanation for the anti-inflammatory properties of this endogenous lectin. Future studies aimed at elucidating the specific role of NF-κB inhibition during Gal-1-induced immunosuppression *in vivo* and the relevance of IKKs and upstream signals in Gal-1 signaling are warranted.

In the present study we show that NF-κB can regulate Gal-1 synthesis and identified many putative κB sequences and at least one functionally active κB sequence in the *LGALS1* gene. In addition, we provide evidence for a novel regulatory function of Gal-1 as an inhibitor of the NF-κB signaling pathway. Interestingly, expression of CD7 which has been shown to serve as a co-receptor during Gal-1-induced T cell death also depends on NF-κB activation (Koh et al., 2008), suggesting that this transcriptional pathway might regulate not only expression of Gal-1, but also expression and activity of its specific glyco-receptors. In addition, this transcriptional regulation also appears to control other members of the galectin family, as regulation of Gal-3 by human T lymphotropic virus I (HTLV-I) was found to be dependent on cyclic AMP-responsive element binding protein and NF-κB induction (Hsu et al., 1996) and a cross-talk between Gal-3 and NF-κB/p65 has been reported in the control of nucling-mediated apoptosis (Liu et al., 2004). However, induction of Gal-1 expression by HTLV-I Tax protein appeared to be independent of NF-κB as it still occurred when NF-κB activation-defective TaxM22 protein was used in reporter assays (Gauthier et al., 2008), suggesting that NF-κB-dependent and -independent mechanisms may operate in the control of Gal-1 expression and functionality.

In multicellular organisms, both normal physiological functions and successful adaptation to environmental changes depend on inducible regulation of gene expression (Pasparakis, 2009). Although NF-κB is a key component in the induction of immunity-related genes, the role of NF-κB as a regulator of Gal-1 expression might go beyond its role in immunity to be generalized to other biological processes including embryogenesis, tissue repair and angiogenesis and could be considered as a regulatory module operating in various biological contexts and signaling processes (Laderach et al., 2010). Moreover, as abnormal NF-κB activation has been identified in numerous pathological conditions such as chronic inflammatory diseases and cancer, rational design of strategies directed to exploit the Gal-1-NF-κB regulatory axis might open new avenues for therapeutic purposes.

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References

Barrionuevo, P., Beigier-Bompadre, M., Ilarregui, J.M., Toscano, M.A., Bianco, G.A., Isturiz, M.A., Rabinovich, G.A., 2007. A novel function for galectin-1 at the crossroad of innate and adaptive immunity: galectin-1 regulates monocyte/macrophage physiology through a nonapoptotic ERK-dependent pathway. J. Immunol. 178, 436–445.

Blaser, C., Kaufmann, M., Muller, C., Zimmermann, C., Wells, V., Mallucci, L., Pircher, H., 1998. Beta-galactoside-binding protein secreted by activated T cells inhibits antigen-induced proliferation of T cells. Eur. J. Immunol. 28, 2311–2319.

Blois, S.M., Ilarregui, J.M., Tometten, M., Garcia, M., Orsal, A.S., Cordo-Russo, R., Toscano, M.A., Bianco, G.A., Kobelt, P., Handjiski, B., Tirado, I., Markert, U.R., Klapp, B.F., Poirier, F., Szekeres-Bartho, J., Rabinovich, G.A., Arck, P.C., 2007. A pivotal role for galectin-1 in fetomaternal tolerance. Nat. Med. 13, 1450–1457.

Cartharius, K., Frech, K., Grote, K., Klocke, B., Haltmeier, M., Klingenhoff, A., Frisch, M., Bayerlein, M., Werner, T., 2005. MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics 21, 2933–2942.

Coornaert, B., Carpentier, I., Beyaert, R., 2009. A20: central gatekeeper in inflammation and immunity. J. Biol. Chem. 284, 8217–8221.

Costas, M., Trapp, T., Pereda, M.P., Sauer, J., Rupprecht, R., Nahmod, V.E., Reul, J.M., Holsboer, F., Arzt, E., 1996. Molecular and functional evidence for in vitro cytokine enhancement of human and murine target cell sensitivity to glucocorticoids. TNF-alpha priming increases glucocorticoid inhibition of TNF-alpha-induced cytotoxicity/apoptosis. J. Clin. Invest. 98, 1409–1416.

Fuertes, M.B., Molinero, L.L., Toscano, M.A., Ilarregui, J.M., Rubinstein, N., Fainboim, L., Zwirner, N.W., Rabinovich, G.A., 2004. Regulated expression of galectin-1 during T-cell activation involves Lck and Fyn kinases and signaling through MEK1/ERK, p38 MAP kinase and p70S6 kinase. Mol. Cell. Biochem. 267, 177–185.

Garin, M.I., Chu, C.C., Golshayan, D., Cernuda-Morollon, E., Wait, R., Lechler, R.I., 2007. Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells. Blood 109, 2058–2065.

Gauthier, S., Pelletier, I., Ouellet, M., Vargas, A., Tremblay, M.J., Sato, S., Barbeau, B., 2008. Induction of galectin-1 expression by HTLV-I Tax and its impact on HTLV-I infectivity. Retrovirology 5, 105–119.

Ghosh, S., Hayden, M.S., 2008. New regulators of NF-kappaB in inflammation. Nat. Rev. Immunol. 8, 837–848.

Green, D.R., Droin, N., Pinkoski, M., 2003. Activation-induced cell death in T cells. Immunol. Rev. 193, 70–81.

Hsu, D.K., Hammes, S.R., Kuwabara, I., Greene, W.C., Liu, F.T., 1996. Human T lymphotropic virus-I infection of human T lymphocytes induces expression of the beta-galactoside-binding lectin, galectin-3. Am. J. Pathol. 148, 1661–1670.

Ilarregui, J.M., Croci, D.O., Bianco, G.A., Toscano, M.A., Salatino, M., Vermeulen, M.E., Geffner, J.R., Rabinovich, G.A., 2009. Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. Nat. Immunol. 10, 981–991.

Imhof, A., Wolffe, A.P., 1998. Transcription: gene control by targeted histone acetylation. Curr. Biol. 8, R422–R424.

Juszczynski, P., Ouyang, J., Monti, S., Rodig, S.J., Takeyama, K., Abramson, J., Chen, W., Kutok, J.L., Rabinovich, G.A., Shipp, M.A., 2007. The AP1-dependent secretion of galectin-1 by Reed Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. Proc. Natl. Acad. Sci. U.S.A. 104, 13134–13139.

Kavurma, M.M., Khachigian, L.M., 2003. Signaling and transcriptional control of Fas ligand gene expression. Cell Death Differ. 10, 36–44.

Koh, H.S., Lee, C., Lee, K.S., Ham, C.S., Seong, R.H., Kim, S.S., Jeon, S.H., 2008. CD7 expression and galectin-1-induced apoptosis of immature thymocytes are directly regulated by NF-kappaB upon T-cell activation. Biochem. Biophys. Res. Commun. 370, 149–153.

Laderach, D.J., Compagno, D., Toscano, M.A., Croci, D.O., Dergan-Dylon, S., Salatino, M., Rabinovich, G.A., 2010. Dissecting the signal transduction pathways triggered by galectin–glycan interactions in physiological and pathological settings. IUBMB Life 62, 1–13.

Lawrence, T., 2009. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb. Perspect. Biol. 1, a001651.

Li, Z.W., Rickert, R.C., Karin, M., 2004. Genetic dissection of antigen receptor induced-NF-kappaB activation. Mol. Immunol. 41, 701–714.

Lin, B., Williams-Skipp, C., Tao, Y., Schleicher, M.S., Cano, L.L., Duke, R.C., Scheinman, R.I., 1999. NF-kappaB functions as both a proapoptotic and anti-apoptotic regulatory factor within a single cell type. Cell Death Differ. 6, 570–582.

Liu, F.T., Rabinovich, G.A., 2010. Galectins: regulators of acute and chronic inflammation. Ann. NY Acad. Sci. 1183, 158–182.

Liu, L., Sakai, T., Sano, N., Fukui, K., 2004. Nucling mediates apoptosis by inhibiting expression of galectin-3 through interference with nuclear factor kappaB signaling. Biochem. J. 380, 31–41.

Motran, C.C., Molinder, K.M., Liu, S.D., Poirier, F., Miceli, M.C., 2008. Galectin-1 functions as a Th2 cytokine that selectively induces Th1 apoptosis and promotes Th2 function. Eur. J. Immunol. 38, 3015–3027.

Nieminen, J., Kuno, A., Hirabayashi, J., Sato, S., 2007. Visualization of galectin-3 oligomerization on the surface of neutrophils and endothelial cells using fluorescence resonance energy transfer. J. Biol. Chem. 282, 1374–1383.

Norling, L.V., Sampaio, A.L., Cooper, D., Perretti, M., 2008. Inhibitory control of endothelial galectin-1 on in vitro and in vivo lymphocyte trafficking. FASEB J. 22, 682–690.

Pasparakis, M., 2009. Regulation of tissue homeostasis by NF-kappaB signalling: implications for inflammatory diseases. Nat. Rev. Immunol. 9, 778–788.

Perillo, N.L., Pace, K.E., Seilhamer, J.J., Baum, L.G., 1995. Apoptosis of T cells mediated by galectin-1. Nature 378, 736–739.

Perone, M.J., Bertera, S., Tawadrous, Z.S., Shufesky, W.J., Piganelli, J.D., Baum, L.G., Trucco, M., Morelli, A.E., 2006. Dendritic cells expressing transgenic galectin-1 delay onset of autoimmune diabetes in mice. J. Immunol. 177, 5278–5289.

Rabinovich, G., Castagna, L., Landa, C., Riera, C.M., Sotomayor, C., 1996. Regulated expression of a 16-kd galectin-like protein in activated rat macrophages. J. Leukoc. Biol. 59, 363–370.

Rabinovich, G.A., Daly, G., Dreja, H., Tailor, H., Riera, C.M., Hirabayashi, J., Chernajovsky, Y., 1999. Recombinant galectin-1 and its genetic delivery suppress collagen-induced arthritis via T cell apoptosis. J. Exp. Med. 190, 385–398.

Rabinovich, G.A., Iglesias, M.M., Modesti, N.M., Castagna, L.F., Wolfenstein-Todel, C., Riera, C.M., Sotomayor, C.E., 1998. Activated rat macrophages produce a galectin-1-like protein that induces apoptosis of T cells: biochemical and functional characterization. J. Immunol. 160, 4831–4840.

Rabinovich, G.A., Ramhorst, R.E., Rubinstein, N., Corigliano, A., Daroqui, M.C., Kier-Joffe, E.B., Fainboim, L., 2002. Induction of allogenic T-cell hyporesponsiveness by galectin-1-mediated apoptotic and non-apoptotic mechanisms. Cell Death Differ. 9, 661–670.

Rabinovich, G.A., Toscano, M.A., 2009. Turning 'sweet' on immunity: galectin–glycan interactions in immune tolerance and inflammation. Nat. Rev. Immunol. 9, 338–352.

Renner, F., Schmitz, M.L., 2009. Autoregulatory feedback loops terminating the NF-kappaB response. Trends Biochem. Sci. 34, 128–135.

Rubinstein, N., Alvarez, M., Zwirner, N.W., Toscano, M.A., Ilarregui, J.M., Bravo, A., Mordoh, J., Fainboim, L., Podhajcer, O.L., Rabinovich, G.A., 2004. Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection; A potential mechanism of tumor-immune privilege. Cancer Cell 5, 241–251.

Santucci, L., Fiorucci, S., Rubinstein, N., Mencarelli, A., Palazzetti, B., Federici, B., Rabinovich, G.A., Morelli, A., 2003. Galectin-1 suppresses experimental colitis in mice. Gastroenterology 124, 1381–1394.

Satelli, A., Rao, U.S., 2011. Galectin-1 is silenced by promoter hypermethylation and its re-expression induces apoptosis in human colorectal cancer cells. Cancer Lett. 301, 38–46.

Stillman, B.N., Hsu, D.K., Pang, M., Brewer, C.F., Johnson, P., Liu, F.T., Baum, L.G., 2006. Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. J. Immunol. 176, 778–789.

Stowell, S.R., Qian, Y., Karmakar, S., Koyama, N.S., Dias-Baruffi, M., Leffler, H., McEver, R.P., Cummings, R.D., 2008. Differential roles of galectin-1 and galectin-3 in regulating leukocyte viability and cytokine secretion. J. Immunol. 180, 3091–3102.

Toscano, M.A., Bianco, G.A., Ilarregui, J.M., Croci, D.O., Correale, J., Hernandez, J.D., Zwirner, N.W., Poirier, F., Riley, E.M., Baum, L.G., Rabinovich, G.A., 2007. Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. Nat. Immunol. 8, 825–834.

Toscano, M.A., Commodaro, A.G., Ilarregui, J.M., Bianco, G.A., Liberman, A., Serra, H.M., Hirabayashi, J., Rizzo, L.V., Rabinovich, G.A., 2006. Galectin-1 suppresses autoimmune retinal disease by promoting concomitant Th2- and T regulatory-mediated anti-inflammatory responses. J. Immunol. 176, 6323–6332.

Vallabhapurapu, S., Karin, M., 2009. Regulation and function of NF-kappaB transcription factors in the immune system. Annu. Rev. Immunol. 27, 693–733.

van der Leij, J., van den Berg, A., Harms, G., Eschbach, H., Vos, H., Zwiers, P., van Weeghel, R., Groen, H., Poppema, S., Visser, L., 2007. Strongly enhanced IL-10 production using stable galectin-1 homodimers. Mol. Immunol. 44, 506–513.

van Kooyk, Y., Rabinovich, G.A., 2008. Protein–glycan interactions in the control of innate and adaptive immune responses. Nat. Immunol. 9, 593–601.

Vereecke, L., Beyaert, R., van Loo, G., 2009. The ubiquitin-editing enzyme A20 (TNFAIP3) is a central regulator of immunopathology. Trends Immunol. 30, 383–391.

Werbajh, S., Nojek, I., Lanz, R., Costas, M.A., 2000. RAC-3 is a NF-kappa B coactivator. FEBS Lett. 485, 195–199.

Zuñiga, E., Rabinovich, G.A., Iglesias, M.M., Gruppi, A., 2001. Regulated expression of galectin-1 during B-cell activation and implications for T-cell apoptosis. J. Leukoc. Biol. 70, 73–79.