Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.
Molecular Pharmacology Fast Forward. Published on May 21, 2004 as doi:10.1124/mol.104.001040

TNFα differentially regulates the expression of pro-inflammatory genes in human airway smooth muscle cells by activation of IFNβ-dependent CD38 pathway

Omar Tliba, Reynold A. Panettieri Jr, Samira Tliba, Timothy F Walseth, and Yassine Amrani.

Pulmonary, Allergy and Critical Care Division, Department of Medicine (OT, RAP, ST, YA), University of Pennsylvania Medical Center, Philadelphia, PA 19104, USA

* Department of Pharmacology (TFW), University of Minnesota Medical School, Minneapolis, MN 55455, USA.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Copyright 2004 by the American Society for Pharmacology and Experimental Therapeutics.

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

a) Runnning Title: Cross-talk between TNFα and CD38/cADPR signaling pathways.

b) Corresponding Author:

Dr. Yassine AMRANI, Pulmonary, Allergy and Critical Care Division, University of Pennsylvania Medical Center, 421 Curie Boulevard, 848 BRB II/III, Philadelphia, PA 19104-6160, USA Phone: 215-573-9851, Fax: 215-573-4469. E-Mail: amrani@mail.med.upenn.edu

c) Number of text pages = 27

Number of figures = 6

Number of references = 41

Word counts:

Abstract = 220

Introduction = 500

Discussion = 1286

d) Abbreviations: ASM, airway smooth muscle: cADPr, cyclic ADPr; 8-Br-cADPr, 8 Bromo-cADPr; GPCR, G-protein coupled receptor; ICAM, intercellular adhesion molecule; IFN, interferon; JAK, Janus activated kinase; NAD, nicotinamide adenine dinucleotide; RT-PCR, reverse transcription-polymerase chain reaction; RANTES, regulated upon activation normal T cell expressed and secreted; STAT, Signal transducers and activators of transcription; TNFR, tumor necrosis factor receptor.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Recent evidence suggests that CD38, an ectoenzyme that converts NAD^+ to cyclic ADP-ribose (cADPr), may play a role in cytokine-induced airway smooth muscle (ASM) cell hyper-responsiveness, a key feature associated with chronic asthma. In the present study, we investigated the major signaling pathways by which TNFα induces CD38 expression as well as its role in regulating gene expression in human ASM cells. Using flow cytometry analyses, TNFα enhanced CD38 expression in a manner that was time (0-24hr), concentration (0.1-40 ng/ml) and protein synthesis (cycloheximide blockade) dependent. A selective agonistic antibody against TNFR1 also augmented CD38 expression while anti-TNFR2 antagonistic antibody did not prevent the TNFα response. Inhibition of the JAK/STAT pathways using a soluble inhibitor ((2-(1,1-Dimethylethyl)-9-fluoro-3,6-dihydro-7H-benz-[h]imidaz[4,5f]isoquinolin-7-one) or with neutralizing antibody against IFNβ completely abrogated TNFα-induced CD38 expression at both protein and mRNA levels. Combining TNFα (0.1 and 1 ng/ml) and IFNβ (100 IU/ml), at concentrations alone that had little effect on CD38 expression, induced a robust synergistic induction of CD38 mRNA and protein levels. 8-bromo-cADPr, a cADPr antagonist, significantly augmented TNFα-induced IL-6 secretion, while RANTES secretion was suppressed. 8-bromo-cADPr, however, did not affect TNFα-induced cell surface expression of ICAM-1. Together, our study is the first to demonstrate that IFNβ-dependent activation of CD38 pathway is a novel component by which TNFα differentially regulates the expression of inflammatory genes in ASM cells.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

CD38 is a bifunctional ectoenzyme with ADP-ribosyl cyclase activity that converts the cellular intermediary metabolite βNAD^+ to cADPr, a calcium (Ca^{2+})-mobilizing second messenger. In addition, CD38 mediates the degradation of cADPr to ADPR through its cADPr hydrolase activity (reviewed in (Lee, 2001)). CD38 expression occurs widely in many mammalian cells including hematopoetic cells, such as B and T lymphocytes, macrophages, as well as resident cells such as pancreas, heart, brain, liver and lung cells, vascular and uterine smooth muscle (reviewed in (Deaglio et al., 2001)). The cellular function of CD38 remains unclear. Early evidence shows that CD38 plays a critical role in insulin release from pancreatic ß cells (Takasawa et al., 1993). Additional studies, using different experimental approaches, such as monoclonal agonistic antibodies, cADPr antagonist 8-Bromo-cADPr (8-Br-cADPr) or CD38-deficient cells demonstrate a role for CD38 in both B and T cell proliferation (Funaro et al., 1997), cytokine production from B and T cells (Deaglio et al., 2003), neutrophil migration (Partida-Sanchez et al., 2001), as well as neurotransmission and cardiac contraction (Higashida et al., 2001). Whether all CD38 cellular effects are mediated via cADPr remains controversial; new evidence shows that CD38 signaling in response to activating antibodies occurs independently of its enzymatic activity (Lund et al., 1999). The nature of these signaling pathways is not known but CD38 ligation can activate multiple molecules such as phospholipase Cγ, phosphatidyl inositol 3-kinase and other tyrosine phosphorylated proteins (Shubinsky and Schlesinger, 1997). Together, these studies show the mechanisms that regulate CD38/cADPr expression and function remain unknown.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

We and others recently showed that the CD38/cADPr pathway represents a novel component in the regulation of Ca^{2+} homeostasis in response to activation of G protein coupled receptors (GPCR) in monocytes (Partida-Sanchez et al., 2004), in arterial SM cells (Ge et al., 2003) and in airway SM cells (Prakash et al., 1998; White et al., 2003). We also found that induction of CD38 expression by TNFα or IL-1β correlated with increases in Ca^{2+} signals to different GPCR agonists (bradykinin, carbachol), an effect that was abrogated by the cADPr antagonist, 8-Br-cADPr (Deshpande et al., 2003). Similar findings were also recently reported in myometrium cells where oxytocin-induced Ca^{2+} responses were enhanced by TNFα, an effect also prevented by 8-Br-cADPr (Barata et al., 2004). Collectively, these data suggest that the modulation CD38/cADPr pathways by inflammatory cytokines may represent one mechanism in the regulation of cell responsiveness to GPCR agonists. We therefore propose that changes in CD38 expression and/or function in ASM, the main effector tissue that regulate the bronchomotor tone, may represent a key mechanism underlying the development of bronchial hyper-responsiveness to GPCRs, a defining feature of asthma.

In this study, we present the first evidence that the induction of CD38 expression by TNFα occurs via transcriptional mechanisms involving the synergistic cooperation of endogenous IFNβ. More importantly, activation of CD38/cADPr pathway by TNFα differentially regulates the expression of inflammatory genes. Our findings shows that, in addition to its recently shown role in promoting airway hyper-responsiveness, CD38/cADPr pathway potentially modulates airway inflammation via the transcriptional regulation of inflammatory genes in ASM cells.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Materials and Methods

Cell culture. Human tracheal tissue culture was obtained from lung transplant donors in accordance with procedures approved by the University of Pennsylvania Committee on Studies Involving Human Beings. The culture of human ASM cells was performed as described elsewhere (Amrani et al., 2001).

Flow cytometry analysis. Flow cytometry was performed as described previously (Amrani et al., 1999). Briefly, adherent cells were washed with PBS, detached by trypsinization (2 min, 37°C) and then washed with Ham's-F12 (10% feotal calf serum) media, centrifuged, and transferred to microfuge tubes (1.5 ml). Following incubation with the mouse anti-human CD38 antibody (2 μg/ml, Santa Cruz Biotechnology, Santa Cruz, CA) and FITC-conjugated goat anti-mouse antibody (Jackson immunoResearch, West Grove, PA), the cells were centrifuged and resuspended in cold PBS in microfuge tubes. Samples were then analyzed using an EPICS XL flow cytometer (Coulter, Hialeah, FL). ICAM-1 expression was assessed using FITC-conjugated mouse anti-human ICAM-1 Ab (10 μg/ml, R&D Systems, Minneapolis, MN). CD38 and ICAM-1 expression was expressed as the fold increases in mean fluorescence intensity over basal (untreated cells).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Western Blot Analysis. Immunoblot analysis for phospho-STAT1 was performed as described previously (Tliba et al., 2003): To ensure equal loading, the membranes were stripped and reprobed with anti-STAT1 (Santa Cruz Biotechnology, Santa Cruz, CA).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis. Total RNA was extracted from human ASM cells using RNeasy mini kit (Qiagen) according to the manufacturer's instructions. RT-PCR reactions were performed using human CD38

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

primers for semi-quantitative analysis as previously described (Deshpande et al., 2003). Each of 35 cycles of the PCR was programmed to carry out denaturation at 94°C for 30 s, primers annealing at 55°C for 45 s, extension at 72°C for 45 s, and a final extension at 72°C for 10 min. The semi-quantitative PCR approach of CD38 mRNA was performed in parallel by investigating human GAPDH mRNA levels with the following primers: 5'-ATGGATGATGATATCGCCGC-3' (sense) and 5'-TTAATGTCACGCACGATTTC-3' (antisense). The intensity of density area was analyzed using a Gel-Pro Analyzer (Silver Spring, MD). The final PCR product was expressed as the ratio of CD38 to GAPDH used for scanning analysis.

Measurement of IL-6 and RANTES Secretion by ASM Cells by ELISA. Confluent ASM cells were growth-arrested by incubating the monolayers in Ham's F12 with 0.1% bovine serum albumin for 24 h and stimulated with TNFα (10ng/ml) for 24 h. The concentration of IL-6 and RANTES in the culture medium was determined by ELISA as described previously (Tliba et al., 2003). To investigate the effect of CD38/cADPr pathway on TNFα induced IL-6 and RANTES expression, the 8-Br-cADPr, a membrane permeant antagonist of cADPr (100 μM) was added 15 min before the addition of TNFα.

Measurement of cADPr levels. cADPr levels were determined by an enzymatic cycling method developed by Graeff et al. (Graeff and Lee, 2002). ASM cultures were treated in the absence or presence of TNFα for 24 hours. The media was then removed by aspiration and 5ml of ice-cold 40 %(v/v) acetonitrile was added (Grob et al., 2003). The cells were scraped from the dish and frozen. After thawing, the cell extracts were sonicated for 20 seconds on ice and centrifuged at 2000xg for 30 minutes to remove

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

precipitated protein. The supernatant was evaporated to dryness using a Savant Speed-Vac concentrator. The cADPr assay depends on the conversion of cADPr to nicotinamide adenine dinucleotide (NAD) using Aplysia ADP-ribosyl cyclase, thus necessitating removal of endogenous NAD before the assay [Graeff, 2002 #2; Graeff, 2003 #3]. To accomplish this, we used reverse phase HPLC to separate cellular NAD from cADPr. The chromatography step utilized a LC18T column (4.6mm x 15cm, Supelco) at a flow rate of 1 ml/min with 10mM KH2PO4, pH 6.0. A gradient from 0-10% methanol in 10mM KH2PO4, pH 6.0 from 6 to 15 minutes was utilized to separate cADPr from NAD. cADPr elutes between 3 and 4 minutes while NAD elutes at approximately 14 minutes. The dried samples were reconstituted in 500µl of 10mM KH2PO4, pH 6.0 and filtered through 0.22µm cellulose acetate centrifugal filters (Spin-x, Corning, Inc) before injection. Fractions containing cADPr (1 ml fractions collected from 2 to 6 minutes) were dried on a Savant Speed-Vac concentrator. These fractions were reconstituted in 200µl of 100mM sodium phosphate, pH 8.0. Forty µl aliquots of the reconstituted fractions were used in the cADPr determination.

Materials and Reagents. Tissue culture reagents and primers used for PCR were obtained from Life Technologies (Grand Island, NY). Human rTNFα was provided by Boehringer Mannheim (Minneapolis, MN). rIFNγ, rIFNβ, rIFNα, and the different antibodies: antagonistic anti-TNFR1, agonistic anti-TNFR1 , neutralizing anti-IFNβ (sheep polyclonal Ab), isotype-matched goat or mouse IgG were all purchased from R&D Systems (Minneapolis, MN). Cycloheximide was purchased from Sigma (St. Louis, MO). The JAK inhibitor (DBI) (2-(1,1-Dimethylethyl)-9-fluoro-3,6-dihydro-7H-benz-[h]imidaz[4,5f]isoquinolin-7-one) was provided by Calbiochem (San Diego, CA). The

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

anti-TNFR2 antagonistic Ab was obtained from Cell Sciences Inc. (Norwood, MA). 8-Br-cADPr was prepared as previously described (Walseth et al., 1997). The Sheep serum was purchased from Jackson ImmunoResearch (West Grove, PA).

Data analysis. Data points from individual assays represent the mean values of triplicate measurements. Significant differences among groups were assessed with ANOVA (Bonferroni-Dunn test) or by t-test analysis, with values of $P < 0.05$ sufficient to reject the null hypothesis for all analyses. Each set of experiments was performed with a minimum of three different human ASM cell lines.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Results

TNFα stimulates CD38 expression and cADPr production in human ASM Cells. In ASM cells stimulated with 10 ng/ml of TNFα for 0-24 h, CD38 expression was increased in a time-dependent manner, with a significant increase of 1.8 ± 0.1 and 4.1 ± 0.5 fold at 12 hr and 24 hr, respectively ($P < 0.01$, Fig. 1A). To determine whether the TNFα effect on CD38 expression was due to protein synthesis, ASM cells were pretreated with the protein synthesis inhibitor cycloheximide (10 μM for 1 h, as described in (Amrani et al., 2000b)). As shown in Figure 1A, cycloheximide completely inhibited TNFα-induced CD38 induction at 24 hr ($P<0.001$). These results suggest that TNFα-induced CD38 expression required *de novo* protein synthesis. In addition, CD38 expression by TNFα (0.1-40 ng/ml, 24 hr) was concentration-dependent with a net fold increase of 4.3 ± 0.2 and 8.2 ± 0.4 at 10 and 40 ng/ml, respectively ($P < 0.01$, Fig. 1B). We also found that the increase in CD38 protein expression was associated with increased levels of cADPr with a net 1600 fmol/mg (n=2, data not shown). For all subsequent experiments, modulation of CD38 expression was examined in ASM cells stimulated with 10 ng/ml of TNFα for 24 h.

CD38 induction by TNFα involves the activation of TNFR1, but not TNFR2. Using a set of agonistic and antagonistic antibodies, we showed that TNFR1 plays an important role in mediating many cellular effects induced by TNFα in ASM cells (Amrani et al., 2001). Incubation of ASM cells with the agonistic antibody to TNFR1 for 24 h stimulates the expression of CD38 with levels similar to those induced by TNFα with a 4.8 ± 0.61 and 5.3 ± 0.45 fold increase over basal, respectively (Fig. 2A). At the same concentration, the isotype-matched antibody had no effect on CD38 levels in basal

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

and TNFα-treated cells. As shown in Figure 2B, neutralizing anti-TNFR2 antibody (20 μg/ml, 1 hr as used in (Amrani et al., 2001)) had little effect on CD38 induction by TNFα. Collectively, these data show that TNFα stimulates CD38 expression in ASM cells mainly by activating TNFR1.

CD38 induction by TNFα requires the autocrine activation of the JAK/STAT pathways. Evidence from our laboratory showed that TNFα can activate the JAK/STAT signaling molecules via the autocrine action of endogenous IFNβ (Tliba et al., 2003)**.** Because type I IFNs can stimulate CD38 expression in other cell types (Bauvois et al., 1999), we next examined whether endogenous IFNβ modulated TNFα-induced CD38 expression and cADPr production. As shown in Figure 3, neutralizing anti-IFNβ antibody significantly suppressed TNFα-induced CD38 expression by more than 85% at both protein (Fig. 3A) and mRNA (Fig. 3B) levels. In contrast, the sheep serum, the antibody diluent, did not have any effect on TNFα-induced CD38 expression. Neutralizing anti-IFNβ antibody also completely abolished TNFα-induced cADPr production at 24 hr (95% inhibition, data not shown).

The involvement of JAK/STAT pathways was confirmed by using the recently described inhibitor of IFN-receptor associated kinases JAK1 and Tyk2, *2-(1,1-Dimethylethyl)-9-fluoro-3,6-dihydro-7H-benz-[h]imidaz[4,5f]isoquinolin-7-one)* (DBI) (Thompson et al., 2002). We found that DBI at 25 nM was effective in inhibiting IFNγ-coupled signaling pathways as shown by the dose-dependent inhibition of IFNγ-induced STAT1 phosphorylation (Fig. 4A). At this particular concentration, we also found that DBI abrogated TNFα-induced increases in CD38 protein (Fig. 4B) and mRNA (Fig. 4C).

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Taken together, these results suggest that the JAK/STAT pathways play an essential role in the transcriptional activation of CD38 gene induced by TNFα.

CD38 induction by TNFα requires the synergistic cooperation of endogenous IFNβ. Although autocrine IFNβ (as shown above) mediated TNFα-induced CD38 expression, the mechanisms by which endogenous IFNβ regulates CD38 expression are not clear. In ASM cells treated with exogenous IFNβ at concentrations believed to be released by TNFα-treated ASM cells (100-IU/ml in (Tliba et al., 2003)), there was no effect on CD38 expression while only a modest stimulatory effect was observed at higher concentrations such as 1000 UI/ml (1.48 ± 0.3 fold increase, n=3, data not illustrated). In addition, another type I IFN, IFNα had no effects on CD38 expression at either 100, 500 or 1000 UI/ml (data not shown). In contrast, there was a significant increase in CD38 levels in ASM cells treated with a combination of ineffective concentrations of TNFα (0.1 and 1 ng/ml as shown in Fig. 1) and IFNβ (100 IU/ml, see above). CD38 expression was increased by 6.1 ± 0.3 and 7.2 ± 0.2 fold when combining 100 IU/ml IFNβ with 0.1 and 1 ng/ml TNFα, respectively (Fig. 5A). RT-PCR analyses of cytokine-treated ASM cells revealed that the synergistic action of TNFα and IFNβ combination was also observed that the mRNA level (Fig. 5B). Together, these results suggest that the induction of CD38 gene by both TNFα and IFNβ is likely to involve cooperative mechanisms that synergistically increase CD38 gene transcription.

cADPr antagonist differentially modulates TNFα-induced gene expression. In previous reports, the use of 8-Br-cADPr, a cell permeant cADPr antagonist (Walseth et al., 1997), allowed us to demonstrate the physiological role of cADPr in ASM cells in the presence or the absence of TNFα (Deshpande et al., 2003; White et al., 2003). Here, we

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

found that 8-Br-cADPr differentially regulates TNFα-induced IL-6 and RANTES secretion while cell surface induction of ICAM-1 expression was not affected. As shown in Figure 6A, 8-Br-cADPr significantly enhanced IL-6 secretion induced by TNFα. IL-6 levels were increased from 3823 $\pm$ 120 to 5231 $\pm$ 180 pg/ml in cells treated with TNFα alone or with 8-Br-cADPr, respectively. In addition, 8-Br-cADPr considerably suppressed by 50% TNFα-induced RANTES secretion. RANTES levels were decreased from 11900 $\pm$ 523 pg/ml to 5843 $\pm$ 120 pg/ml in cells treated with TNFα alone or with 8-Br-cADPr, respectively (Figure 6B). Interestingly, 8-Br-cADPr had no effect on ICAM-1 induction by TNFα treated cells (Figure 6C). These data suggest that activation CD38/cADPr pathway differentially regulates TNFα-induced expression of inflammatory genes in ASM cells.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

Recent reports from our laboratories suggest that CD38 expression and activation by inflammatory cytokines in ASM may represent a key molecular mechanism for the development of bronchial hyper-responsiveness, a defining feature of asthma (Amrani et al., 2000a). The present work provides the unique demonstration that activation of CD38/cADPr pathways by TNFα occurs via the autocrine action of endogenous IFNβ and differentially regulates the expression of different inflammatory genes.

Growing evidence shows that binding of TNFα to TNFR1 in ASM activates multiple signaling pathways and genes that may be critical to the pathogenesis of asthma (Amrani et al., 2000a). TNFR1 engagement promotes bronchial hyper-responsiveness by altering Ca^{2+} homeostasis in ASM, the main effector tissue that regulates bronchomotor tone (Amrani et al., 2000a; Hunter et al., 2003; Parris et al., 1999). Activation of TNFR1 in ASM may also regulate airway inflammation, another major characteristic of asthmatics, by promoting the secretion and/or expression of different inflammatory molecules including cytokines and chemokines (Amrani et al., 2001; Amrani et al., 2000b). In the present study, we found that TNFα binding to TNFR1 stimulated the expression of CD38 in human ASM cells, in a manner that was concentration (inducible at 10ng/ml), time (detectable at 12 hr) and protein synthesis (blockade by cycloheximide) dependent. Although a similar finding has been also described in human myometrium cells where induction of CD38 protein by TNFα was associated with an increased cADPr cyclase activity (Barata et al., 2004), the nature of TNFα receptor type involved in CD38 expression was not investigated. This is an important question since ASM cells express both TNFR1 and TNFR2 that mediate some TNFα-induced cellular responses such as

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

RANTES expression (Amrani et al., 2001). Other reports performed in Hela and 293 cells also showed the contribution of both TNFR2 and TNFR1 in TNFα-induced cellular function including apoptotic responses (Fotin-Mleczek et al., 2002) or antiviral activities (Chan et al., 2003). Our present findings suggest that activation of CD38 gene solely involves TNFR1-associated signaling molecules, although the nature of these pathways remains unknown.

The recently described JAK inhibitor DBI (Thompson et al., 2002) and present study, completely blocked the induction of CD38 expression induced by TNFα, suggesting the contribution of the JAK/STAT pathway. In a recent report, we showed that TNFα, via the autocrine action of IFNβ, activates different members of the JAK/STAT pathways including the kinases JAK1 and Tyk2, as well as the transcription factors STAT1 and STAT2 (Tliba et al., 2003). The observations that neutralizing antibodies completely blocked increased CD38 expression (at both protein and mRNA levels) and activity (cADPr production) suggest that TNFα effect on CD38 occured at the transcriptional level via the secretion of endogenous IFNβ. Even though CD38 gene induction by TNFα has also been observed in other excitable cell types such as human myometrium (Barata et al., 2004) as well as mesangial cells (Yusufi et al., 2001), our study is the first demonstration of a role of the autocrine action of IFNβ in TNFα-induced CD38/cADPr both expression and activation. The effect of exogenous Type I IFNs on CD38 induction remains controversial; CD38 is induced by Type I IFNs in some cell types, such as leukemic B cells and resting B lymphocytes (Bauvois et al., 1999; Galibert et al., 1996) but not in others such as hairy leukaemia cells (Hassan et al., 1991). We also found that exogenous IFNγ (Deshpande et al., 2003), or IFNβ alone (present study) failed

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

to effectively increase CD38 levels; however, IFNβ strongly enhanced CD38 steady state mRNA and protein levels when combined with ineffective concentrations of TNFα (0.1 and 1 ng/ml, Figure 5). Interestingly, the magnitude of CD38 expression induced by the combination of subthreshold concentrations of TNFα and 100 IU/ml exogenous IFNβ was greater than that induced by the effective concentration of TNFα alone (Fig. 3). This apparent discrepancy in CD38 induction may be due to the delayed effects of endogenous IFNβ on TNFα-induced CD38 expression, which is only secreted and functional after 3 hr (Tliba et al., 2003). Alternatively, exogenous and endogenous IFNβ may also manifest different cellular effects due to access to different cellular compartments. A previous article showed that endogenous IFNβ induced intracellular signaling events without being secreted and consequently may act differently from exogenous IFNβ (Rousseau et al., 1995). Our observation in Figure 5 underscores the cooperative action between TNFα and IFNβ receptor-coupled signaling pathways to achieve maximal CD38 gene expression. Interestingly, CD38 promoter contains binding sites for multiple transcription factors including IRF-1 (Ferrero and Malavasi, 1997), known to be activated by TNFα or type I IFNs (Tliba et al., 2003). It is plausible that CD38 induction by TNFα and IFNβ may occur by increasing promoter activation through a synergistic action of IRF-1 with other transcription factors including NF-κB or STAT1 as described previously (Hiroi and Ohmori, 2003; Saura et al., 1999). The transcriptional cooperation observed between subeffective concentrations of TNFα and IFNβ may also involve an increased STAT phosphorylation either on tyrosine residues that augment STAT activity or on serine residues required for maximal transcription of the target gene. We found that 100 IU/ml IFNβ-induced STAT1 phosphorylation on two key residues, tyrosine 701 and Serine 727,

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

were not affected by subeffective concentrations of TNFα (Amrani, unpublished observation), suggesting that additional mechanisms may explain the synergistic induction of CD38 gene by both cytokines. Such transcriptional cooperation may explain the functional synergism induced by the combination of TNFα and IFNβ found in other cells such as the inhibition of cell proliferation in human tumor-derived cell lines and murine macrophages (Hamilton et al., 1996; Onozaki et al., 1988), and in the enhancement of the anti-viral activity in human fibroblasts (Reis et al., 1989). Whether the failure of TNFα to promote CD38 expression in monocytes (Musso et al., 2001) or in endothelial cells (Favaloro, 1993) is due to the lack to induce IFNβ in these cells is an interesting hypothesis that remains to be explored.

Our laboratories showed that CD38 enzymatic activity was increased by 3.7 fold in TNFα-treated cells when compared to unstimulated cells (Deshpande et al., 2003). We now confirm that the increased CD38 expression by TNFα is associated with a 1.37 fold increase in the production of cADPr. The functional consequence of cADPr accumulation on ASM function has not been completely investigated, but our earlier studies showed that cADPr-dependent pathways are playing a critical role in mediating cytokine effects on agonist-evoked Ca^{2+} signals (Deshpande et al., 2004; Deshpande et al., 2003). We now show that ASM cells treated with TNFα in the presence of 8-Br-cADPr, a membrane permeant antagonist of cADPr (Walseth et al., 1997; White et al., 2003), released more IL-6 and produced less RANTES. Moreover, the effect of 8-Br-cADPr seems to be gene-specific since no effect was observed in TNFα-induced ICAM-1 expression. Interestingly, CD38 ligation using monoclonal antibodies can regulate the expression of different inflammatory mediators including IL-2, IL-10, IL-6 in a variety of

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

lymphoid cells such as T cells, B cells, NK cells (reviewed in (Deaglio et al., 2001)). Our study, however, is the first to implicate CD38/cADPr pathways in the differential induction of inflammatory cytokines in response to a physiological stimulus TNFα. The fact that autocrine IFNβ (Tliba et al., 2003) and 8 -Br-cADPr elicited similar modulatory effects on TNFα-inducible inflammatory genes (increased IL-6, reduced RANTES) suggests that the transcriptional cooperation induced by endogenous IFNβ and TNFα occurs, at least in part, via the activation of CD38/cADPr pathways.

Our data shows for the first time that activation of CD38/cADPr pathways by TNFα involves the autocrine action of IFNβ and differentially regulates the expression of inflammatory genes in human ASM cells. Further studies are needed to delineate the molecular mechanisms underlying CD38 expression by TNFα as well as the CD38-associated signaling pathways that regulate inflammatory gene expression.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Acknowledgments

The authors thank Mary McNichol for assistance in the preparation of the manuscript.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

References

Amrani Y, Ammit AJ and Panettieri RA, Jr. (2001) Tumor Necrosis Factor Receptor (TNFR) 1, but Not TNFR2, Mediates Tumor Necrosis Factor-alpha -Induced Interleukin-6 and RANTES in Human Airway Smooth Muscle Cells: Role of p38 and p42/44 Mitogen-Activated Protein Kinases. *Mol Pharmacol* **60**:646-655.

Amrani Y, Chen H and Panettieri RJ (2000a) Activation of tumor necrosis factor receptor 1 in airway smooth muscle: a potential pathway that modulates bronchial hyper-responsiveness in asthma? *Respir Res* **1**:49-53.

Amrani Y, Lazaar AL, Hoffman R, Amin K, Ousmer S and Panettieri RA, Jr. (2000b) Activation of p55 Tumor Necrosis Factor-alpha Receptor-1 Coupled to Tumor Necrosis Factor Receptor-Associated Factor 2 Stimulates Intercellular Adhesion Molecule-1 Expression by Modulating a Thapsigargin-Sensitive Pathway in Human Tracheal Smooth Muscle Cells. *Mol Pharmacol* **58**:237-245.

Amrani Y, Lazaar AL and Panettieri RA, Jr. (1999) Up-Regulation of ICAM-1 by Cytokines in Human Tracheal Smooth Muscle Cells Involves an NF-{kappa}B-Dependent Signaling Pathway That Is Only Partially Sensitive to Dexamethasone. *J Immunol* **163**:2128-2134.

Barata H, Thompson M, Zielinska W, Han YS, Mantilla CB, Prakash YS, Feitoza S, Sieck G and Chini EN (2004) The role of cyclic-ADP-ribose-signaling pathway in oxytocin-induced Ca2+ transients in human myometrium cells. *Endocrinology* **145**:881-9.

Bauvois B, Durant L, Laboureau J, Barthelemy E, Rouillard D, Boulla G and Deterre P (1999) Upregulation of CD38 gene expression in leukemic B cells by interferon types I and II. *J Interferon Cytokine Res* **19**:1059-1066.

Chan FK, Shisler J, Bixby JG, Felices M, Zheng L, Appel M, Orenstein J, Moss B and Lenardo MJ (2003) A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. *J Biol Chem* **278**:51613-51621.

Deaglio S, Capobianco A, Bergui L, Durig J, Morabito F, Duhrsen U and Malavasi F (2003) CD38 is a signaling molecule in B-cell chronic lymphocytic leukemia cells. *Blood* **102**:2146-2155.

Deaglio S, Mehta K and Malavasi F (2001) Human CD38: a (r)evolutionary story of enzymes and receptors. *Leuk Res* **25**:1-12.

Deshpande DA, Dogan S, Walseth TF, Miller SM, Amrani Y, Panettieri J, Reynold A and Kannan MS (2004) Modulation of calcium signaling by IL-13 in human airway smooth muscle: role of CD38/cADPR pathway. *Am. J. Respir. Cell Mol. Biol.*:2003-0313OC.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Deshpande DA, Walseth TF, Panettieri RA and Kannan MS (2003) CD38-cyclic ADP-ribose-mediated Ca2+ signaling contributes to airway smooth muscle hyperresponsiveness. *Faseb J*:FASEB Journal Express Article 10.1096/fj.02-0450fje.

Favaloro EJ (1993) Differential expression of surface antigens on activated endothelium. *Immunol Cell Biol* **71 (Pt 6)**:571-581.

Ferrero E and Malavasi F (1997) Human CD38, a leukocyte receptor and ectoenzyme, is a member of a novel eukaryotic gene family of nicotinamide adenine dinucleotide+-converting enzymes: extensive structural homology with the genes for murine bone marrow stromal cell antigen 1 and aplysian ADP-ribosyl cyclase. *J Immunol* **159**:3858-3865.

Fotin-Mleczek M, Henkler F, Samel D, Reichwein M, Hausser A, Parmryd I, Scheurich P, Schmid JA and Wajant H (2002) Apoptotic crosstalk of TNF receptors: TNF-R2-induces depletion of TRAF2 and IAP proteins and accelerates TNF-R1-dependent activation of caspase-8. *J Cell Sci* **115**:2757-2770.

Funaro A, Morra M, Calosso L, Zini MG, Ausiello CM and Malavasi F (1997) Role of the human CD38 molecule in B cell activation and proliferation. *Tissue Antigens* **49**:7-15.

Galibert L, Burdin N, de Saint-Vis B, Garrone P, Van Kooten C, Banchereau J and Rousset F (1996) CD40 and B cell antigen receptor dual triggering of resting B lymphocytes turns on a partial germinal center phenotype. *J Exp Med* **183**:77-85.

Ge ZD, Zhang DX, Chen YF, Yi FX, Zou AP, Campbell WB and Li PL (2003) Cyclic ADP-ribose contributes to contraction and Ca2+ release by M1 muscarinic receptor activation in coronary arterial smooth muscle. *J Vasc Res* **40**:28-36.

Graeff R and Lee HC (2002) A novel cycling assay for cellular cADP-ribose with nanomolar sensitivity. *Biochem J* **361**:379-84.

Grob MK, O'Brien K, Chu JJ and Chen DD (2003) Optimization of cellular nucleotide extraction and sample preparation for nucleotide pool analyses using capillary electrophoresis. *J Chromatogr B Analyt Technol Biomed Life Sci* **788**:103-11.

Hamilton JA, Whitty GA, Kola I and Hertzog PJ (1996) Endogenous IFN-alpha beta suppresses colony-stimulating factor (CSF)-1-stimulated macrophage DNA synthesis and mediates inhibitory effects of lipopolysaccharide and TNF-alpha. *J Immunol* **156**:2553-2557.

Hassan IB, Lantz M and Sundstrom C (1991) Effect of alpha-IFN on cytokine-induced antigen expression and secretion of TNF, LT and IgM in HCL. *Leuk Res* **15**:903-910.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Higashida H, Hashii M, Yokoyama S, Hoshi N, Chen XL, Egorova A, Noda M and Zhang JS (2001) Cyclic ADP-ribose as a second messenger revisited from a new aspect of signal transduction from receptors to ADP-ribosyl cyclase. *Pharmacol Ther* **90**:283-96.

Hiroi M and Ohmori Y (2003) The transcriptional coactivator CREB-binding protein cooperates with STAT1 and NF-kappa B for synergistic transcriptional activation of the CXC ligand 9/monokine induced by interferon-gamma gene. *J Biol Chem* **278**:651-660.

Hunter I, Cobban HJ, Vandenabeele P, MacEwan DJ and Nixon GF (2003) Tumor Necrosis Factor-alpha-Induced Activation of RhoA in Airway Smooth Muscle Cells: Role in the Ca(2+) Sensitization of Myosin Light Chain(20) Phosphorylation. *Mol Pharmacol* **63**:714-721.

Lee HC (2001) Physiological functions of cyclic ADP-ribose and NAADP as calcium messengers. *Annu Rev Pharmacol Toxicol* **41**:317-345.

Lund FE, Muller-Steffner HM, Yu N, Stout CD, Schuber F and Howard MC (1999) CD38 signaling in B lymphocytes is controlled by its ectodomain but occurs independently of enzymatically generated ADP-ribose or cyclic ADP-ribose. *J Immunol* **162**:2693-2702.

Musso T, Deaglio S, Franco L, Calosso L, Badolato R, Garbarino G, Dianzani U and Malavasi F (2001) CD38 expression and functional activities are up-regulated by IFN-gamma on human monocytes and monocytic cell lines. *J Leukoc Biol* **69**:605-612.

Onozaki K, Urawa H, Tamatani T, Iwamura Y, Hashimoto T, Baba T, Suzuki H, Yamada M, Yamamoto S, Oppenheim JJ and et al. (1988) Synergistic interactions of interleukin 1, interferon-beta, and tumor necrosis factor in terminally differentiating a mouse myeloid leukemic cell line (M1). Evidence that interferon-beta is an autocrine differentiating factor. *J Immunol* **140**:112-119.

Parris JR, Cobban HJ, Littlejohn AF, MacEwan DJ and Nixon GF (1999) Tumour necrosis factor-alpha activates a calcium sensitization pathway in guinea-pig bronchial smooth muscle. *J Physiol* **518**:561-569.

Partida-Sanchez S, Cockayne DA, Monard S, Jacobson EL, Oppenheimer N, Garvy B, Kusser K, Goodrich S, Howard M, Harmsen A, Randall TD and Lund FE (2001) Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nat Med* **7**:1209-16.

Partida-Sanchez S, Iribarren P, Moreno-Garcia ME, Gao JL, Murphy PM, Oppenheimer N, Wang JM and Lund FE (2004) Chemotaxis and calcium responses of phagocytes to formyl peptide receptor ligands is differentially regulated by cyclic ADP ribose. *J Immunol* **172**:1896-1906.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Prakash YS, Kannan MS, Walseth TF and Sieck GC (1998) Role of cyclic ADP-ribose in the regulation of [Ca2+]i in porcine tracheal smooth muscle. *Am J Physiol* **274**:C1653-1660.

Reis LF, Ho Lee T and Vilcek J (1989) Tumor necrosis factor acts synergistically with autocrine interferon-beta and increases interferon-beta mRNA levels in human fibroblasts. *J Biol Chem* **264**:16351-16354.

Rousseau V, Cremer I, Lauret E, Riviere I, Aguet M and De Maeyer E (1995) Antiviral activity of autocrine interferon-beta requires the presence of a functional interferon type I receptor. *J Interferon Cytokine Res* **15**:785-9.

Saura M, Zaragoza C, Bao C, McMillan A and Lowenstein CJ (1999) Interaction of interferon regulatory factor-1 and nuclear factor kappaB during activation of inducible nitric oxide synthase transcription. *J Mol Biol* **289**:459-71.

Shubinsky G and Schlesinger M (1997) The CD38 lymphocyte differentiation marker: new insight into its ectoenzymatic activity and its role as a signal transducer. *Immunity* **7**:315-24.

Takasawa S, Tohgo A, Noguchi N, Koguma T, Nata K, Sugimoto T, Yonekura H and Okamoto H (1993) Synthesis and hydrolysis of cyclic ADP-ribose by human leukocyte antigen CD38 and inhibition of the hydrolysis by ATP. *J Biol Chem* **268**:26052-26054.

Thompson JE, Cubbon RM, Cummings RT, Wicker LS, Frankshun R, Cunningham BR, Cameron PM, Meinke PT, Liverton N, Weng Y and DeMartino JA (2002) Photochemical preparation of a pyridone containing tetracycle: a Jak protein kinase inhibitor. *Bioorg Med Chem Lett* **12**:1219-1223.

Tliba O, Tliba S, Da Huang C, Hoffman RK, DeLong P, Panettieri RA, Jr. and Amrani Y (2003) Tumor Necrosis Factor {alpha} Modulates Airway Smooth Muscle Function via the Autocrine Action of Interferon {beta}. *J. Biol. Chem.* **278**:50615-50623.

Walseth TF, Aarhus R, Gurnack ME, Wong L, Breitinger HG, Gee KR and Lee HC (1997) Preparation of cyclic ADP-ribose antagonists and caged cyclic ADP-ribose. *Methods Enzymol* **280**:294-305.

White TA, Kannan MS and Walseth TF (2003) Intracellular calcium signaling through the cADPR pathway is agonist specific in porcine airway smooth muscle. *Faseb J* **17**:482-484.

Yusufi AN, Cheng J, Thompson MA, Dousa TP, Warner GM, Walker HJ and Grande JP (2001) cADP-ribose/ryanodine channel/Ca2+-release signal transduction pathway in mesangial cells. *Am J Physiol Renal Physiol* **281**:F91-102.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Footnotes:

a) This work was supported by National Institutes of Health Grants NIH DA11806 (TFW), 2R01-HL55301 (RAP) and HL67663 (RAP) and by American Lung Association Grant RG-062-N (to YA). Yassine Amrani is a Parker B. Francis Fellow in Pulmonary Research.

b) Dr. Yassine AMRANI, Pulmonary, Allergy and Critical Care Division, University of Pennsylvania Medical Center, 421 Curie Boulevard, 848 BRB II/III, Philadelphia, PA 19104-6160, USA Phone: 215-573-9851, Fax: 215-573-4469. E-Mail: amrani@mail.med.upenn.edu

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Figure legends

Fig. 1. TNFα induces CD38 expression. ASM cells were incubated with 10 ng/ml of TNFα for the indicated time (*left panel: A*) or for 24 h at the indicated concentrations (*right panel: B*). ASM cells were also treated with cycloheximide (CHX, 10 μg/ml) added 1 h prior TNFα for 24 hr. CD38 expression was assessed by flow cytometry as described in *Materials and Methods*. The results are expressed as mean of fold increase over basal ± SEM of three separate experiments. *$P < 0.05$ and **$P < 0.05$ compared with untreated cells. $^{\#}P < 0.05$ compared with cells treated with TNFα alone.

Fig. 2: TNFR1 mediates TNFα-induced CD38 expression. A, human ASM cells were incubated for 24 h with either TNFα (10 ng/ml), agonistic antibody to TNFR1 (5 μg/ml) or the isotype-matched antibody. B, human ASM cells were incubated for 24 h with TNFα (10 ng/ml) in the presence or the absence of neutralizing antibody to TNFR2 or the isotype-matched antibody (10 μg/ml, 1 h prior cytokine). CD38 expression was then assessed by flow cytometry as described in *Materials and Methods*. The results are expressed as mean of fold increase over basal ± SEM of three separate experiments. *$P < 0.05$ compared with untreated cells.

Figure 3. TNFα, via the autocrine action of secreted IFNβ, induces CD38 gene expression**.** Cells were stimulated for 24 h with TNFα (10 ng/ml) in the presence or absence of neutralizing anti-IFNβ (5 μg/ml) or sheep serum (control) added 15 min before. A, CD38 protein expression was analyzed by flow cytometry as described in *Materials and Methods*. The results are expressed as mean of fold increase over basal ± SEM of three separate experiments. *$P < 0.05$ compared with untreated cells. $^{\#}P < 0.05$ compared with cells treated with TNFα alone. B, Cells were lysed, total mRNA was

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

isolated, and RT-PCR was performed as described in *Materials and Methods* using specific primers for CD38. The lower panel is the scanning densitometric of the representative gel (upper panel) with each value normalized over the mean density of the corresponding GAPDH PCR products.

Figure 4. TNFα induced CD38 expression via the JAK/STAT pathways. A, human ASM cells were treated IFNγ (100UI/ml) for 15 min in the presence or absence of the indicated concentration of JAK inhibitor (called DBI) added 30 min before. Cells were then lysed, and nuclear extracts were prepared and assayed for the phosphorylated and nonphosphorylated forms of STAT1 by immunoblot analysis as described in *Materials and Methods*. Results are representative of three separate blots. B-C, Human ASM cells were stimulated with 10 ng/ml of TNFα in the presence or absence of the JAK inhibitor I (DBI) (25 nM) or DMSO 0.1% added 30 min before. B, CD38 protein expression was analyzed by flow cytometry as described in *Materials and Methods*. The results are expressed as mean of fold increase over basal ± SEM of three separate experiments. $^{*}P < 0.05$ compared with untreated cells. $^{\#}P < 0.05$ compared with cells treated with TNFα alone. C, Representative agarose gel showing the CD38 PCR products stained with ethidium bromide. Cells were lysed, total mRNA was isolated, and RT-PCR was performed as described in *Materials and Methods* using specific primers for CD38. Below is the scanning densitometric of the representative gel with each value normalized over the mean density of the corresponding GAPDH PCR products.

Figure 5. Synergistic activation of CD38 gene by TNFα and IFNβ. Cells were stimulated for 24 h with the indicated concentration of TNFα and IFNβ (100 U/ml) alone or in combination. A. CD38 protein expression was analyzed by flow cytometry as described

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

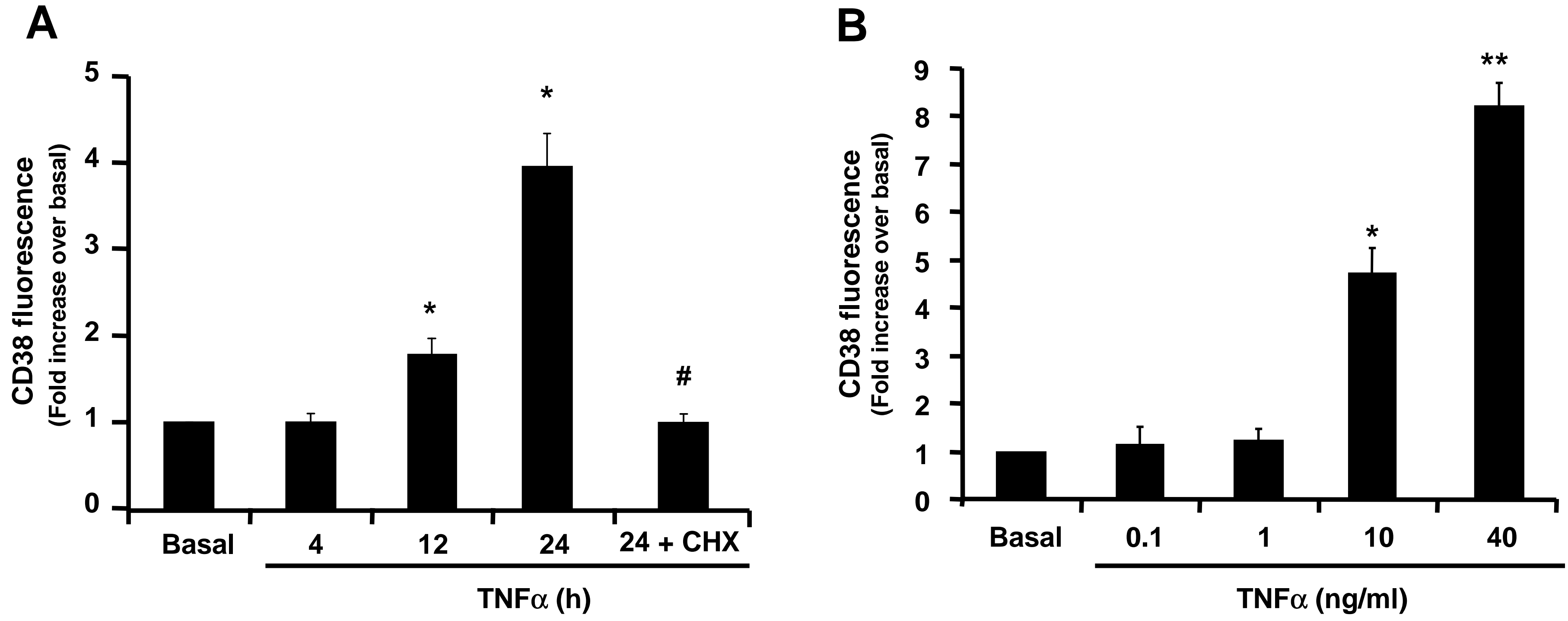
Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

in *Materials and Methods*. The results were expressed as mean of fold increase over basal ± SEM of three separate experiments. *$P < 0.05$ compared with untreated cells. B, Representative agarose gel showing the CD38 PCR products stained with ethidium bromide. Cells were lysed, total mRNA was isolated, and RT-PCR was performed as described in *Materials and Methods* using specific primers for CD38. Below is the scanning densitometric of the representative gel with each value normalized over the mean density of the corresponding GAPDH PCR products.

Figure 6. 8-Br-cADPr differentially regulates TNFα-induced expression of IL-6, RANTES and ICAM-1. Cells were stimulated for 24 h with TNFα (10 ng/ml) in the presence or absence of 8-Br-cADPr (100 μM, added 15 min). Secretion of IL-6 (A) and RANTES (B) or expression of ICAM-1 (C) were analyzed as described in *Materials and Methods*. Values shown are mean ± SEM of three separate experiments. *$P < 0.05$ compared with untreated cells. #$P < 0.05$ compared with cells treated with TNFα alone. NS, non-significant when compared with cells treated with TNFα alone.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

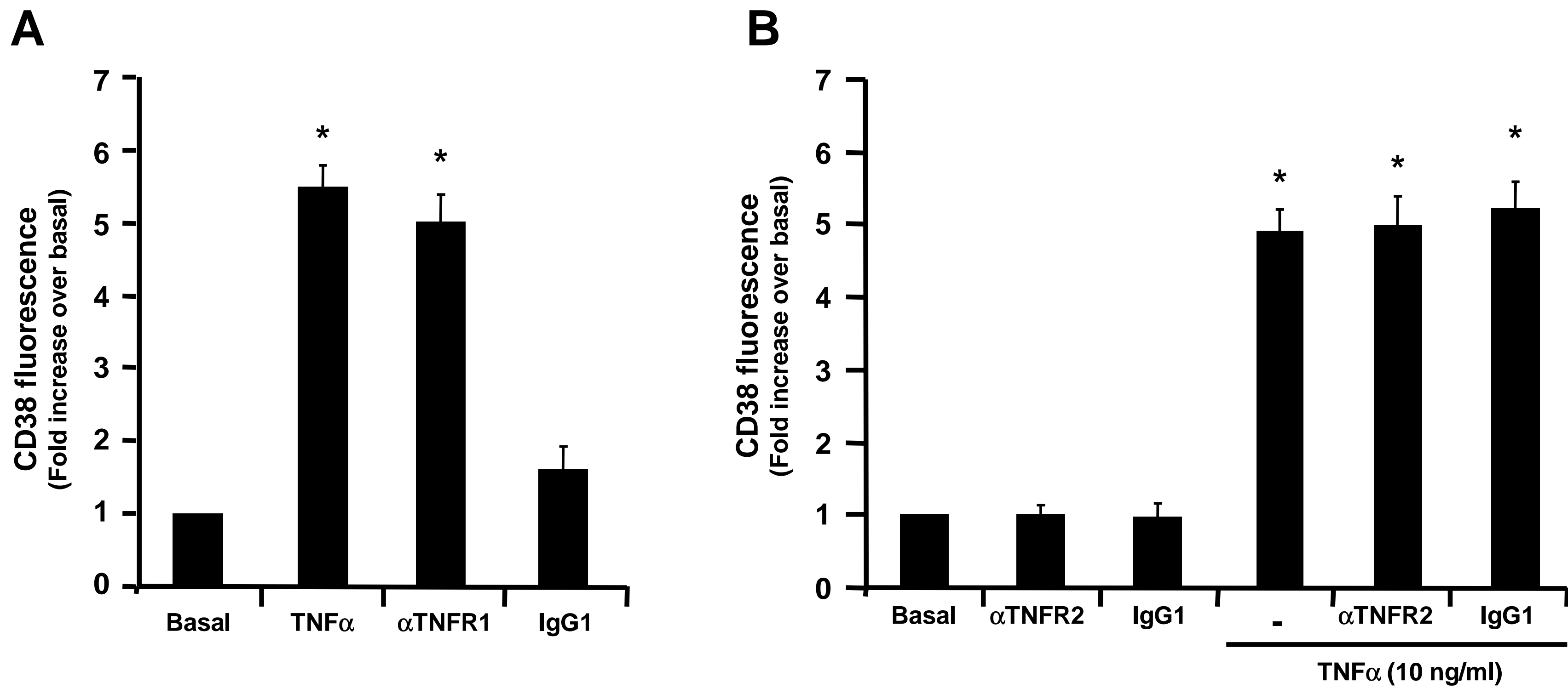
Figure 1



Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

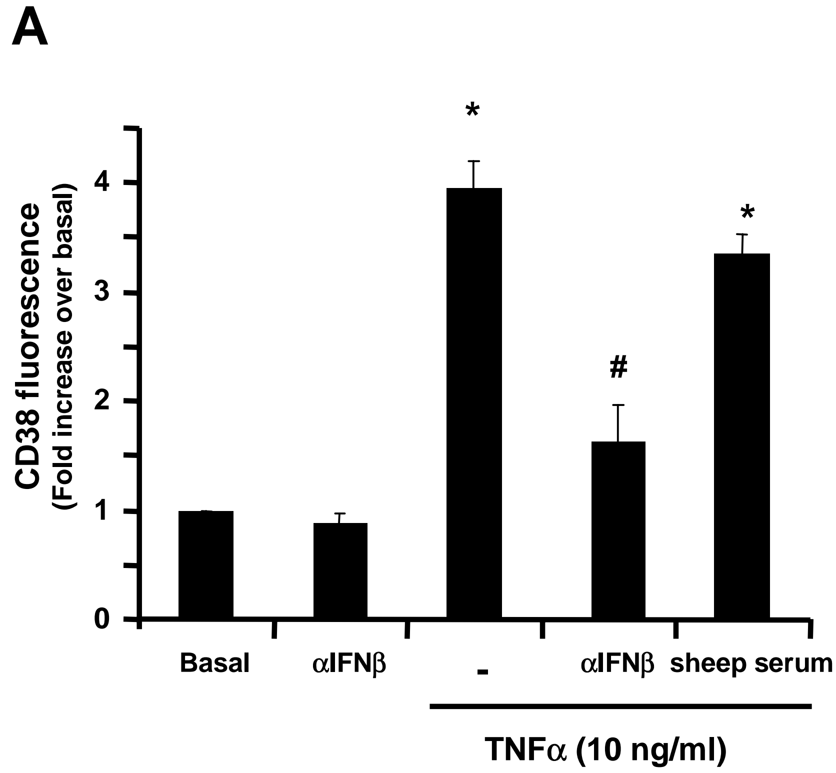
Figure 2

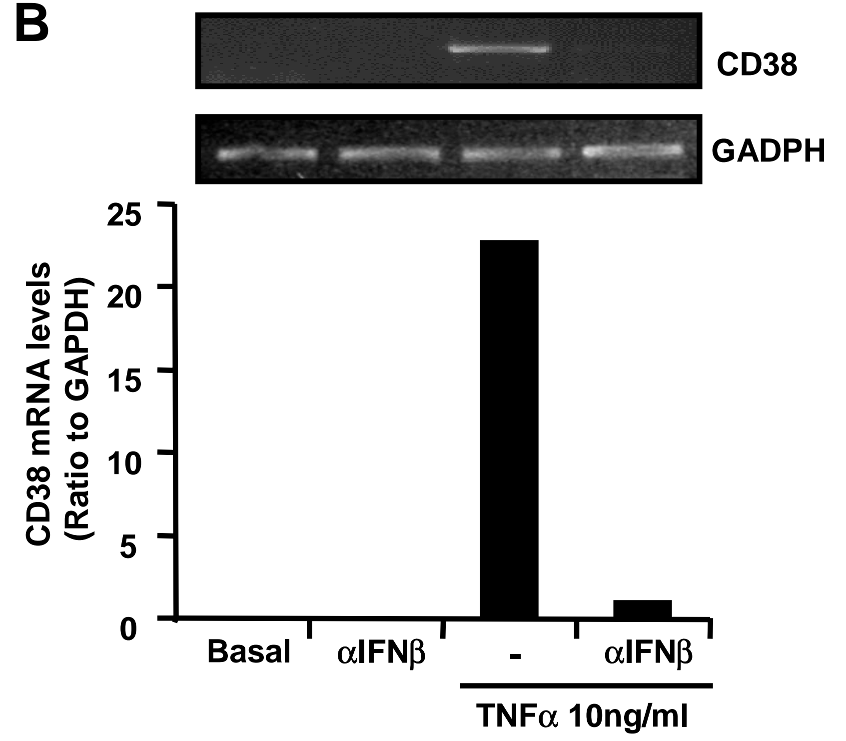


Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Figure 3

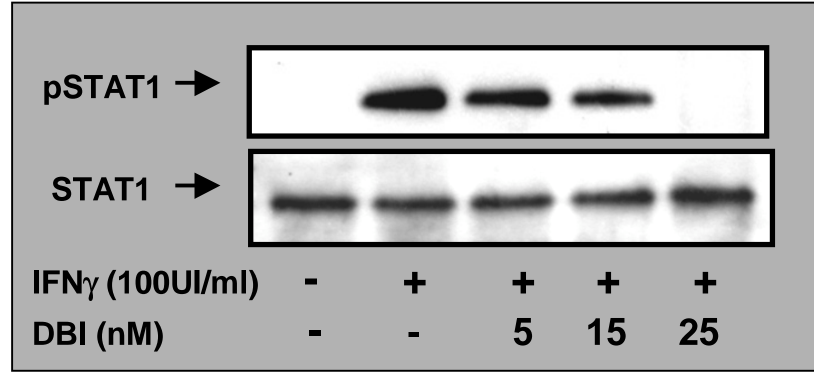


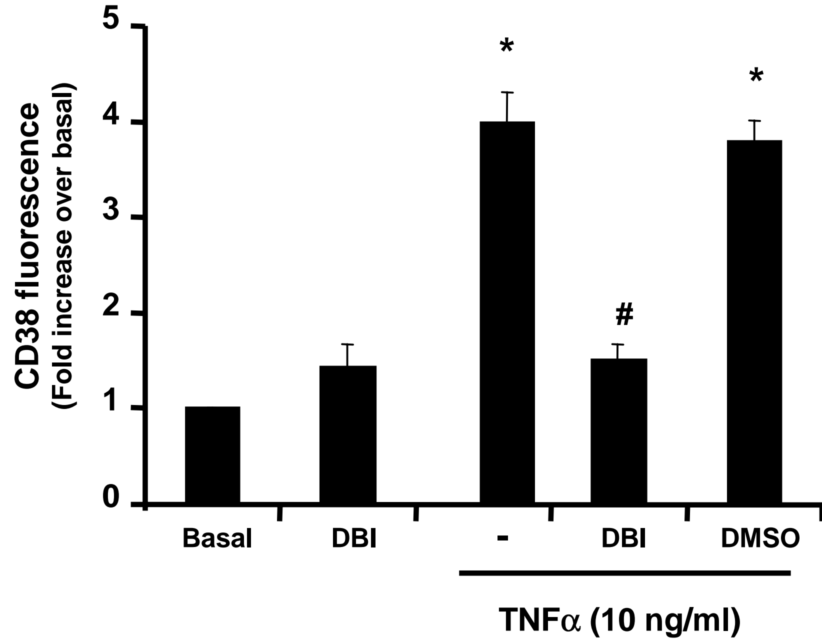


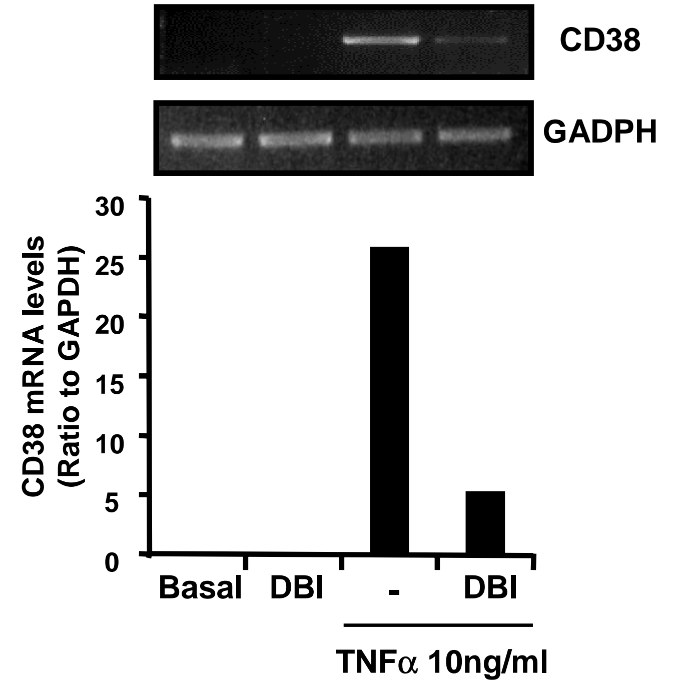
Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Figure 4



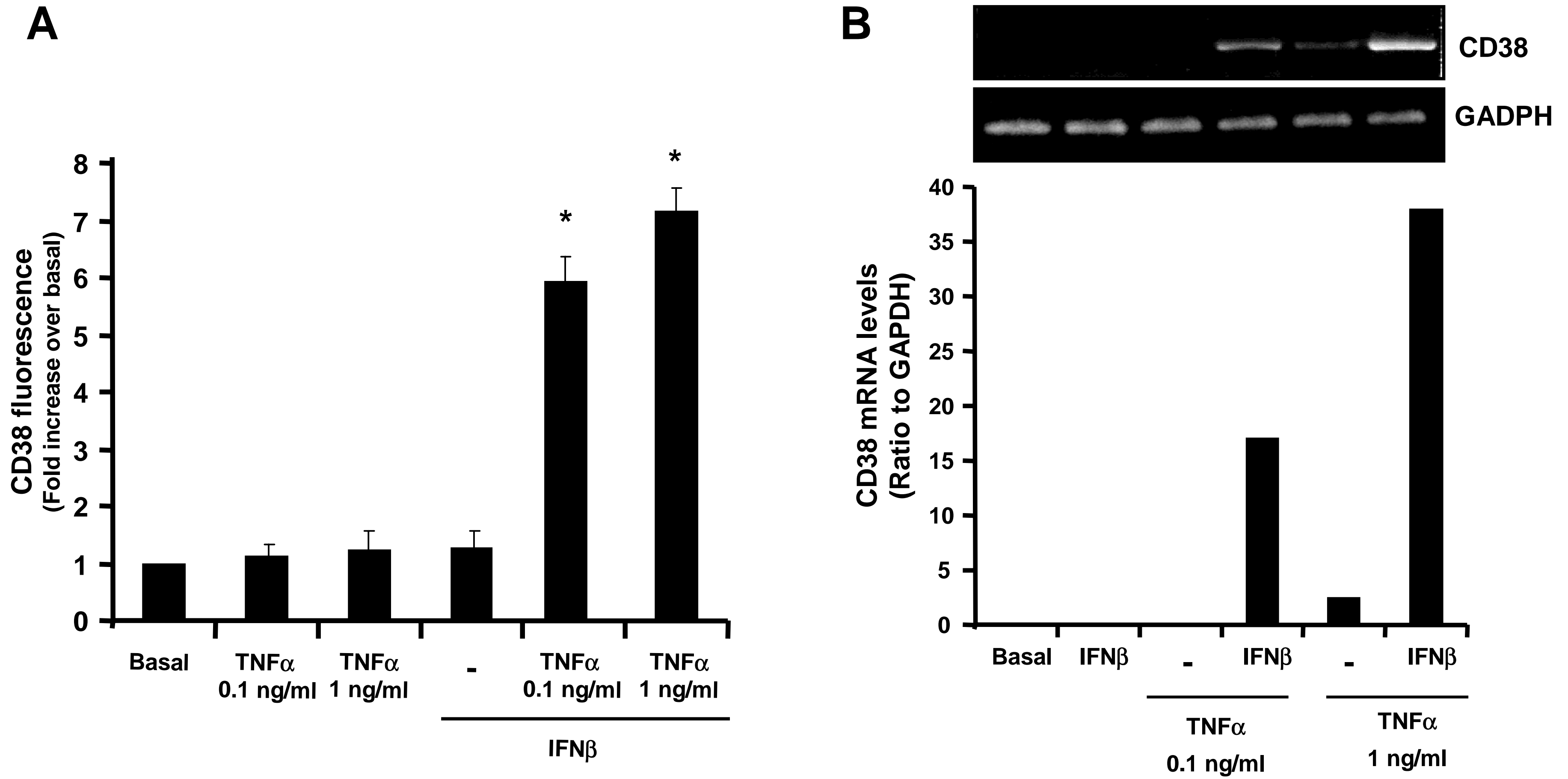




Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

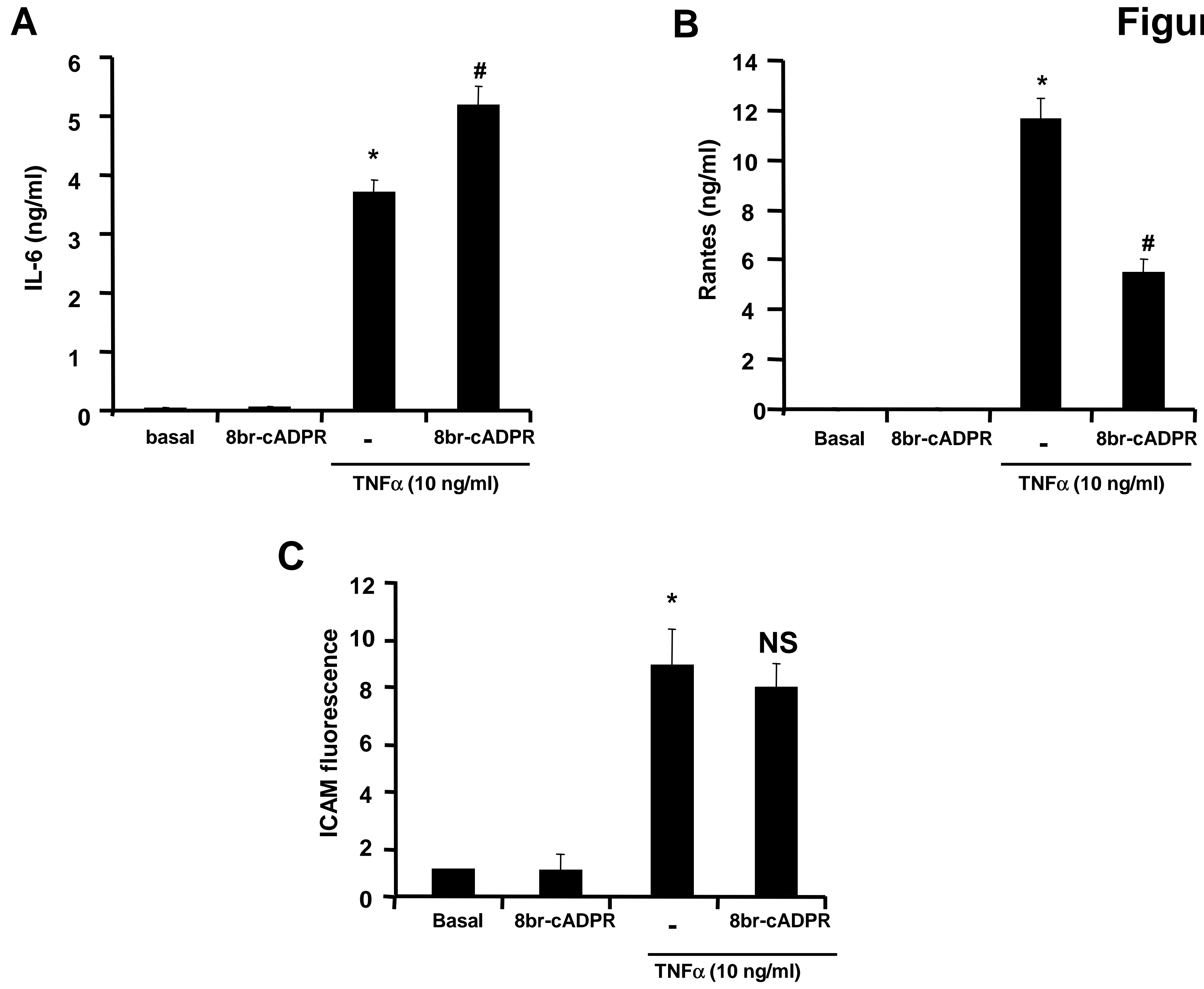
Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024

Figure 5



Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024



Molecular Pharmacology Fast Forward. Published on May 21, 2004 as DOI: 10.1124/mol.104.001040
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 17, 2024