HHS Public Access

Author manuscript

J Cell Physiol. Author manuscript; available in PMC 2017 May 01.

Published in final edited form as:

J Cell Physiol. 2016 May ; 231(5): 992–1000. doi:10.1002/jcp.25216.

Non-hematopoietic β-arrestin1 confers protection against experimental colitis

Taehyung Lee[1], **Eunhee Lee**[1], **David Arrollo**[1], **Peter C. Lucas**[2], and **Narayanan Parameswaran**[1]

[1]Department of Physiology, Michigan State University, East Lansing, Michigan, USA

[2]Department of Pathology, University of Pittsburgh, Pennsylvania, USA

Abstract

β-Arrestins are multifunctional scaffolding proteins that modulate G protein-coupled receptor (GPCR)-dependent and -independent cell signaling pathways in various types of cells. We recently demonstrated that β-arrestin1 (β-arr1) deficiency strikingly attenuates dextran sodium sulfate (DSS)-induced colitis in mice. Since DSS-induced colitis is in part dependent on gut epithelial injury, we examined the role of β-arr1 in intestinal epithelial cells (IECs) using a colon epithelial cell line, SW480 cells. Surprisingly, we found that knockdown of β-arr1 in SW480 cells enhanced epithelial cell death via a caspase-3-dependent process. To understand the in vivo relevance and potential cell type-specific role of β-arr1 in colitis development, we generated bone marrow chimeras with β-arr1 deficiency in either the hematopoietic or non-hematopoietic compartment. Reconstituted chimeric mice were then subjected to DSS-induced colitis. Similar to our previous findings, β-arr1 deficiency in the hematopoietic compartment protected mice from DSS-induced colitis. However, consistent with the role of β-arr1 in epithelial apoptosis in vitro, non-hematopoietic β-arr1 deficiency led to an exacerbated colitis phenotype. To further understand signaling mechanisms, we examined the effect of β-arr1 on TNF-α-mediated NFκB and MAPK pathways. Our results demonstrate that β-arr1 has a critical role in modulating ERK, JNK and p38 MAPK pathways mediated by TNF-α in IECs. Together, our results show that β-arr1-dependent signaling in hematopoietic and non-hematopoietic cells differentially regulates colitis pathogenesis and further demonstrates that β-arr1 in epithelial cells inhibits TNF-α-induced cell death pathways.

Introduction

β-Arrestins (β-arr1 and β-arr2) are ubiquitously present intracellular scaffolding proteins known for their classic role in G protein-coupled receptor (GPCR) desensitization. Recent studies have demonstrated that β-arrestins are required not only for GPCR desensitization but also for a number of G protein-dependent as well as GPCR-independent functions (Whalen et al., 2011). Consistent with their broad role in cell signaling processes, β-arrestins

Corresponding author: Narayanan Parameswaran BVSc., PhD., Department of Physiology and Division of Pathology, 567 Wilson Road, #2201, Biomedical Physical Sciences Building, Michigan State University, East Lansing, MI 48824, USA, Ph: 517-884-5115, narap@msu.edu.

Authors declare no conflict of interest.

have been shown to be important in many disease processes. It is now clear that β-arrestins are critical cell signaling regulators in a number of inflammatory diseases including sepsis (Fan et al., 2010; Sharma et al., 2013), arthritis (Li et al., 2013; Li et al., 2011), autoimmune encephalomyelitis (Shi et al., 2007; Tsutsui et al., 2008), and inflammatory bowel diseases (Lee et al., 2013; Sharma et al., 2015; Zeng et al., 2015).

Inflammatory bowel diseases (IBDs) are complex and multifactorial, characterized by chronic inflammation and epithelial injury at various sites in the gastrointestinal (GI) tract. A variety of cells in the intestinal tract including cells of the hematopoietic (e.g. immune) and non-hematopoietic (e.g. epithelial) compartments have been shown to play a critical role in maintaining gut homeostasis (Maloy and Powrie, 2011). In particular, the intestinal epithelium composed of a single layer of epithelial cells provides a critical barrier for preventing invasion of commensal bacteria and pathogens from the intestinal lumen to the underlying tissue. A precise balance between epithelial cell proliferation and apoptosis is essential for the maintenance of this barrier function. In fact, recent studies demonstrate that dysregulated epithelial cell turnover and apoptosis contribute to the perpetuation of chronic intestinal inflammation (Ramachandran et al., 2000). Furthermore, cytokines such as TNF-α released during intestinal inflammation have been shown to be associated with epithelial cell apoptosis, culminating in the pathogenesis of IBD (Begue et al., 2006; Goretsky et al., 2012; Marjoram et al., 2015).

In previous studies we demonstrated that β-arr1 is an important mediator of DSS-induced colitis (Lee et al., 2013). Using a whole body knockout (KO) of β-arr1, we found that β-arr1 deficiency protects mice from colitis and this was associated with a marked decrease in IL-6 production in the KO mice compared to the wild types. Although we attributed this initially to regulation of β-arr1-mediated pathways in the immune compartment, we soon discovered that the role of β-arr1 in disease processes is highly context dependent. In support of this, we found that the β-arr1 KO mice that are protected from colitis are in fact more susceptible to polymicrobial sepsis. This negative role of β-arr1 in sepsis is mediated via the non-hematopoietic compartment (Sharma et al., 2014). Dysregulation in the turnover of IECs is a critical pathogenic factor in both sepsis and colitis in human disease and mouse models (Husain and Coopersmith, 2003; Kim et al., 2010; Peterson and Artis, 2014). Importantly, pathogenesis of DSS-induced colitis in mice is dependent in large part on gut epithelial cell turnover. Therefore, we hypothesized that β-arr1 is an important regulator of epithelial cell turnover in the intestine. To test this we utilized an in vitro model of epithelial cell line with normal or knockdown levels of β-arr1 as well as an in vivo model of β-arr1 chimeric mice with KO of β-arr1 either in the hematopoietic or non-hematopoietic compartment. Contrary to our expectations in the IBD model but similar to the role of β-arr1 in sepsis, our results here demonstrate that β-arr1 in the non-hematopoietic cells is an important negative regulator of colitis development. This appears to be in part associated with the role of β-arr1 as an inhibitor of intestinal epithelial apoptosis possibly via modulation of TNF-α-induced MAPK and caspase-3 pathways.

Materials and Methods

Cell culture and reagents

Human intestinal epithelial cell line, SW480 was purchased from the American Type Culture Collection (ATCC, VA, USA). Cells were cultured in RPMI1640 medium with 10% FBS, penicillin and streptomycin at 37 °C and 5% CO_2 incubator. Recombinant human TNF-α and cycloheximide were purchased from PeproTech (Rocky Hill, NJ) and Sigma-Aldrich (St. Louis, MO), respectively.

Animals

β-Arr1 KO mice (Conner et al., 1997)(kindly provided by Dr. Lefkowitz, Duke University) and WT mice maintained at Michigan State University have been previously described (Lee et al., 2013; Porter et al., 2010; Sharma et al., 2014). All animals were housed in a specific-pathogen-free facility maintained at 22–24°C with a 12-h light–dark cycle and were given mouse chow and water *ad libitum*. All experiments were performed with age- and sex-matched mice between 8–10 weeks of age unless indicated otherwise. All animal procedures were approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC) and conformed to NIH guidelines.

Generation of chimeric mice

Chimeric mice were generated using lethal irradiation and bone marrow (BM) reconstitution as described by us before (Sharma et al., 2014). Briefly, recipient mice were irradiated with a total dose of 11 gy, delivered in two fractions 3 h apart to reduce gastrointestinal (GI) toxicity and injected with 5×10^6 bone marrow cells from donor, 12 h after the second fraction of irradiation. Mice were put on water with antibiotics (sulfamethoxazole and trimethoprim, Hi-Tech Pharmacal Co., NY) for 4 weeks with every week change of antibiotics and were used for experiments after 14 weeks.

DSS-induced colitis

Chimeric mice were subjected to 2% (wt/vol) DSS (reagent-grade DSS salt; molecular mass = 36–50 KD; MP Biomedicals) in drinking water for 6–7 days with every 2 days change of fresh DSS, followed by regular water for the indicated time points. Body weight was monitored daily until mice were sacrificed.

Histopathology

Distal colonic tissues were collected from mice subjected to DSS. Tissues were fixed in 10% formalin, embedded in paraffin, then sectioned and stained with hematoxylin and eosin (H&E) and Ki67. The degree of inflammation on longitudinal sections of the colon was scored by a Board certified pathologist (PCL) in a blinded manner as described before (Lee et al., 2013). For each colon section, a determination was made of the percentage of the mucosal surface area involved with severe damage (defined as complete mucosal ulceration/total epithelial denudation and associated marked inflammation), moderate damage (defined as partial crypt damage and distortion/partial epithelial destruction and associated modest

inflammation), or minimal damage (defined as slight/focal cryptitis or essentially normal, undistorted mucosa).

As a second, complementary measure of inflammatory involvement, longitudinal sections were scored using a well-established composite scoring system as described before (Ostanin et al., 2009). This system involves assigning an overall histologic activity score that represents the sum of the following features: degree of inflammation in lamina propria (score 0–3); 2) goblet cell loss (score 0–2); 3) abnormal crypts (score 0–3); 4) presence of crypt abscesses (score 0–1); 5) mucosal erosion and ulceration (score 0–1); 6) submucosal spread or transmural involvement (score 0–3); 7) number of neutrophils counted at ×40 magnification (score 0–4). By totaling values from the seven parameters, the overall histologic activity score (total histopathological score) can range from 0–17.

siRNA transfection

Smartpool Control and β-arr1 siRNAs were obtained from Dharmacon (GE Dharmacon, Lafayette, CO). Transfections of siRNAs were performed with 50 nM siRNA using the HiPerfect transfection reagent (Qiagen, Valencia, CA). The cells were transfected and analyzed for knockdown 48 h after transfection.

Proliferation assay

Forty-eight hours after transfection of SW480 cells (with control or β-arr1 siRNA), transfected cells were washed twice with PBS. Phenol red-free culture media was added and then the cells were untreated or treated with TNF-α (20 ng/ml) for the indicated times. Cell proliferation was assessed by measuring cellular DNA content using the CyQUANT Cell Proliferation Assay Kit (Life Technologies, Grand Island, NY) according to the manufacture's protocols. Fluorescence was measured on a Tecan Spectra FluorPlus fluorescence plate reader with excitation at 485 nm and emission at 530 nm.

Apoptosis assay

SW480 cells were transfected with control or β-arr1 siRNA. Forty-eight hours after transfection, cells were serum starved for overnight and then pretreated with cyclohexamide (CHX, 10 μg/ml) for 1 hr prior to TNF-α (20 ng/ml) for the indicated times. After treatment for the indicated time points, cells were trypsinized with 0.25% trypsin-EDTA. The cells were then stained using the Annexin V-FITC Apoptosis Detection Kit (eBioscience, San Diego, CA) according to the manufacture's instructions. After incubation, the apoptotic cells were measured by flow cytometry using the LSR II (BD Biosciences, San Jose, CA).

ROS detection

Untreated and treated cells were labeled with CM-H_2DCFDA (Life Technologies, Grand Island, NY) at final concentration of 10 μM according to manufacture's protocols. Intracellular ROS levels were measured by LSRII flow cytometer (BD Biosciences, San Jose, CA).

Western Blot analysis—Control and β-arr1 siRNA transfected cells were untreated or treated with TNFα for the indicated time points. Cells were then lysed using lysis buffer and

cell lysates prepared and subjected to SDS-PAGE gel electrophoresis followed by Western blotting as described before (Lee et al., 2013; Patial et al., 2011). Blots were probed with antibodies for caspase-3, caspase-8, pERK1/2, pNFκBp65, pJNK and pP38 and scanned using Licor's Odyssey as described (Patial et al., 2011).

Statistical analysis—All values are represented as mean±SEM. Each "N" represents individual mouse. Data were analyzed and statistics performed using GRAPHPAD PRISM software (La Jolla, California). The student's t-test (two-tailed) was used to compare mean values between two experimental groups and ANOVA with post-Sidak for comparing more than two groups (one or two way ANOVA depending on the experimental data). Any deviations from these tests are indicated in the figure legends. P value of less than 0.05 was considered significant.

Results

β-Arrestin-1 inhibits epithelial cell death

Pathogenesis of DSS-induced colitis in part depends on mechanisms that affect either proliferation or apoptosis of epithelial cells (Araki et al., 2010; Okayasu et al., 1990). To understand if the protective effect of β-arr1 deficiency in colitis in part can be explained by β-arr1's role in epithelial cell turnover, we used an in vitro model of β-arr1 deficient IECs. For this we utilized human colonic epithelial cell line SW480 cells transfected with either control or β-arr1 siRNA. Western blotting revealed that β-arr1 is knocked down by over 90% (Figure. 1A). Knockdown of β-arr1 did not affect β-arr2 expression (Supplementary Figure 2A). Given the pathogenic role of TNF-α in colitis development, we focused on the role of TNF-α-induced epithelial cell proliferation and apoptosis in the presence or absence of β-arr1 knockdown. As shown in Fig 1B, β-arr1 knockdown did not cause a significant change in TNF-α-induced proliferation of SW480 cells as determined by proliferation assay. To examine if TNF-α affected SW480 apoptosis, we treated these cells (control siRNA and β-arr1 siRNA transfected cells) with TNF-α in the presence of cyclohexamide (CHX). CHX was used to inhibit TNF-α-NFκB-dependent synthesis of anti-apoptotic proteins (Salaun et al., 2010). To assess apoptosis, we performed flow cytometry to examine Annexin-V and PI staining. Interestingly and contrary to our prediction, the Annexin-V positive, and PI negative population (early apoptotic cells) was significantly enhanced in β-arr1-knockdown cells compared to control cells, especially at 24 hours post-treatment (Fig 1C and D). Interestingly we also observed late apoptotic cells (Annexin-V and PI double positive) cells to be enhanced in the β-arr1 knockdown cells at 48 hours post-treatment, suggesting overall increase in early and late apoptotic cells in the β-arr1 knockdown cells at these respective time points. To confirm that this effect is caspase-dependent, we examined caspase-3 and -8 activation by Western blotting. As shown in Fig 2, caspase-3 activation was significantly increased in β-arr1-knockdown cells compared to control cells 24 h after TNF-α treatment, and the enhanced activation was retained up to 36 h post treatment. Compared to caspase-3, caspase-8 (upstream to caspase-3) activation was similar between control and β-arr1 siRNA transfected groups (Fig 2). These observations indicate that β-arr1 is a critical inhibitor of TNF-α-stimulated-caspase-3-cell death pathway in SW480, intestinal epithelial cell line. Together, these results also suggest that, contrary to prediction, β-arr1 in epithelial cells may

be protective and therefore knockdown or KO of β-arr1 in epithelial cells may render these cells more prone for apoptosis and therefore worse intestinal inflammation.

β-Arrestin1 in non-hematopoietic compartment protects mice from DSS-induced colitis

To begin to understand the physiological relevance of these cell culture findings, and to examine the role of β-arr1 in the non-hematopoietic system, we generated bone marrow chimeras in which the donors and/or the recipients were either wild type (WT) (CD45.1) or β-arr1-deficient (CD45.2). The chimeric mice were found to have >95% donor-derived leukocytes in the blood and >94% donor-derived leukocytes in the spleen, as determined using flow cytometry by distinguishing between 45.1 and 45.2 alleles (Supplementary figure 1). Fourteen weeks after bone marrow transplantation, the different groups of mice were subjected to 2% DSS treatment (in drinking water) over a period of 6–7 days, followed by drinking water. Body weight was monitored during this time period. Consistent with our previous findings using whole body β-arr1 KO, β-arr1 deficiency in hematopoietic cells (KO➔WT) protected mice from colitis-induced weight loss compared to the WT➔WT group (Figure. 3A). Unexpectedly however, mice lacking β-arr1 in the non-hematopoietic compartment (WT➔KO) exhibited more severe weight loss than that observed in WT➔WT mice. Consistent with the exacerbated weight loss in these mice, gross colon pathology (as assessed by colon length) as well as histology showed evidence of excessive damage in the non-hematopoeitic β-arr1 KO compared to the WT mice (Figure. 3B and C). Hematoxylin/eosin (H&E) staining of the colon sections from both chimeric mice (WT➔WT and WT➔KO) demonstrated chronic mucosal ulceration, marked leukocyte infiltration, and a loss of epithelial architecture, but these features were significantly exacerbated in chimeric mice lacking β-arr1 in non-hematopoietic cells. Taken together, these findings indicate that β-arr1 signaling in non-hematopoietic compartment exerts a previously unappreciated protective effect in mice subjected to DSS treatment. Intriguingly, these results are consistent with the *in vitro* effects of β-arr1 in SW480 cell line. Although we attempted to demonstrate *in vivo* epithelial cell death in the chimeric mice, we were unsuccessful likely due to the time points of harvest. Regardless, taken together with the in vitro effect of β-arr1 in cell death in colonic epithelial cell line, our results suggest that β-arr1 in the non-hematopoietic compartment (likely epithelial cells) is protective during colitis.

Mechanisms of β-arr1 regulation of intestinal epithelial cell death

Our *in vitro* and *in vivo* results so far demonstrate that β-arr1 in IECs might play an important and previously unappreciated protective role in colitis development. To begin to understand the signaling mechanisms of these effects, we examined the upstream regulators of TNF-α-induced caspase-3 activation in SW480 cells. We initially focused on reactive oxygen species since TNF-α-induced ROS production has been linked to caspase-3 activation and cell death in murine IECs (Babu et al., 2012; Jin et al., 2008). To assess ROS production, we measured intracellular ROS production by using CM-H_2DCFDA and assessed by flow cytometry. As shown in Fig 4, although ROS production was increased after TNF-α treatment for the indicated times, β-arr1 knockdown did not change the levels of ROS production in SW480 cells (Figure 4 A and B), suggesting that β-arr1 regulation of caspase-3 activation was likely independent of ROS. To further understand the signaling mechanisms by which β-arr1 might regulate TNF-α-induced caspase-3 activation and

subsequent apoptosis, we focused on MAPK and NFκB signaling pathways. β-Arr1 has been shown to regulate both MAPK (ERK, JNK and p38) and NFκB pathways in different cell types (Luttrell and Gesty-Palmer, 2010) and these pathways have been implicated in TNF-α-induced apoptosis in various cell types including IECs (Assi et al., 2006; Bavaria et al., 2014; Seidelin et al., 2013; Yan et al., 2001). To test this, we treated SW480 cells (control and β-arr1 knockdown) with TNF-α and examined the phosphorylation status of these pathways. We found that while β-arr1-knockdown did not affect TNF-α-induced pNFκBp65 activation, phosphorylation of ERK1/2 (Extracellular signal-regulated kinase1/2) by TNF-α was significantly inhibited in β-arr1-knockdown cells. Interestingly, phospho-p38 and phospho-JNK (c-Jun N-terminal kinase) levels were significantly enhanced in the knockdown cells after TNF-α treatment, suggesting that differential modulation of the MAPK signaling by β-arr1 could lead to higher apoptosis of IECs (Figure 5A and B). Together these results suggest that TNFα-mediated MAPK pathways are modulated by β-arr1 and this role of β-arr1 in the non-hematopoietic system likely plays a key inhibitory role in DSS-induced colitis development.

Discussion

β-Arrestin1 and 2 were originally discovered and described for their role as negative regulators of G protein-dependent signaling in the GPCR signaling pathway. Further research has demonstrated that these molecules by virtue of being scaffolding proteins can interact with a number of effector proteins in the GPCR signaling pathway. Thus, β-arrestins play key roles in many processes in the GPCR life-cycle and signaling including receptor internalization, recycling, degradation as well as activation of a variety of signaling pathways such as MAPK and NFκB (Luttrell and Gesty-Palmer, 2010; Reiter et al., 2012). In addition to being a critical regulator of GPCR signaling, β-arrestins are also important in modulating signaling pathways from a number of other receptor classes such as TNFR (Kawamata et al., 2007; Kook et al., 2014), TLRs (Fan et al., 2007; Porter et al., 2010; Wang et al., 2006), tyrosine kinase receptors (Hupfeld and Olefsky, 2007), and cytokine receptors (Kovacs et al., 2009). Because of these various roles, β-arrestins are known to regulate basic biological processes such as proliferation and apoptosis in a number of different systems. Indeed, even though β-arr1 and 2 knockout mice are viable, and overtly normal, β-arr1/2 double knockout mice are embryonically lethal due to the requirement of at least one of the β-arrestins for normal fetal development. Thus it is not surprising that β-arr1/2 double KO mouse embryo fibroblasts have altered apoptosis upon GPCR stimulation (Revankar et al., 2004). More recent studies have implicated β-arrestin-dependent signaling in GPCR-induced cell death or survival, mediated by stimulation of metabotropic glutamate receptor 1 (mGluR1) (Emery et al., 2010), urotensin II receptor (Esposito et al., 2011), angiotensin II receptor 1A (Kim et al., 2009), glucagon-like peptide-1 Gs-coupled receptor (GLP-1) (Quoyer et al., 2010), D1 and 2 dopamine receptors (Chen et al., 2009; Chen et al., 2004; Manning and Cantley, 2007), and platelet-activating factor receptor (Xu et al., 2013). Recent evidence also demonstrates β-arrestin-dependent signaling contributes to cell death or survival in non-GPCR signaling pathways such as TLR4 (Li et al., 2010), transforming growth factor beta receptor type 3 (TGFβRIII) (Chen et al., 2003), and TNF-α receptor (TNFR) (Kook et al., 2014). β-arr1 has also been shown to mediate apoptosis in MEFs via

both extrinsic (stimulation with TNF-α/CHX) and intrinsic (genotoxic drug etoposide) pathways. A β-arr1 fragment (1–380) (cleaved by caspase-8) was also shown to directly interact with tBID in the mitochondria, leading to enhanced release of cytochrome c and subsequent activation of caspase-3 and apoptosis (Kook et al., 2014). These differential roles of β-arrestins in different cell types is rather not surprising given the broad cell-type and receptor-specific roles of β-arrestins. In our studies using colonic epithelial cell line β-arr1 inhibited apoptosis in response to TNFα treatment. Whether this role of β-arr1 in epithelial cell apoptosis could explain the phenotype observed in the non-hematopoietic β-arr1 knockout mice in DSS-induced colitis will be the subject of future studies.

Unlike other death receptors, TNFR1 signaling has been demonstrated to mostly mediate inflammatory genes. It is established that pro-survival/pro-inflammatory pathway activates NFκB and MAPK via signaling complex I, which transcribes anti-apoptotic genes such as cFLIP, cIAP, XIAP, TRAF2, A20, and Bcl-xL. Thus, the blockade of complex I-mediated signaling pathway using inhibitors such as CHX is required to induce apoptosis via TNFR1 stimulation in most cell types including epithelial cells (Bhattacharya et al., 2003). This inhibition has been demonstrated to promote TNF-α-induced apoptosis by blocking the synthesis of cFLIP, an inhibitor of caspase-8 (Kreuz et al., 2001). Recent studies have indicated that CHX not only inhibits TNF-α-mediated cFLIP expression, it also increases its degradation, leading to caspase activation and increased apoptosis (Wang et al., 2008). $cFLIP_L$ is also known to be targeted to degradation via the E3 ubiquitin ligase Itch under TNF-α/CHX treatment conditions (Chang et al., 2006). Since β-arrestins have been shown to interact with various E3 ligases (Kommaddi and Shenoy, 2013), and since caspase-8 is a direct activator of caspase-3, we initially hypothesized that β-arr1 might be involved in caspase-8 activation. However β-arr1 knockdown did not affect activation of caspase-8 suggesting that the effect of β-arr1 on TNF-α-induced apoptosis in SW480 cells is in part dependent on caspase-3 but not caspase-8.

MAP kinases are serine and threonine protein kinases and include ERK, JNK and p38 MAPK families. They regulate a wide range of cellular processes including cell survival and apoptosis. Even though their role in apoptosis has been shown to be more complex, stimulation of ERK pathway has been linked to cell survival whereas activation of JNK and p38 MAPK has been linked to apoptosis (Hung et al., 2003) (Yan et al., 2004). In our present studies, TNFα-induced ERK1/2 phosphorylation appears to be mediated via β-arr1. In contrast to the ERK pathway, knockdown of β-arr1 enhanced TNF-α-induced JNK and p38 phosphorylation suggesting differential regulation of ERK and JNK/p38 pathways by β-arr1. Previous studies have demonstrated that JNK pathway can activate caspase-3 (Bhattacharya et al., 2004; Jin et al., 2006; Ray et al., 2005). In future studies we will determine the mechanisms by which β-arr1 differentially regulates ERK and JNK/P38 pathways as well as determine the role of these pathways in caspase-3 activation and apoptosis in colitis.

In conclusion, our studies here demonstrate a previously unappreciated protective role of β-arr1 in intestinal epithelial cell apoptosis and its potential role in the development of colitis. Future studies will focus on the in vivo mechanisms by which β-arr1 in the non-hematopoietic cells inhibit intestinal inflammation and further delineate the mechanisms by

which hematopoietic β-arr1 mediates colitis development as well as determine how β-arr1 in the two cellular compartments oppose colitis pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

We thank Dr. Robert Lefkowitz for kindly providing us the β-arrestin1 knockout mice. We thank the university lab animal resources for taking excellent care of our animals, and the Histopathology laboratory for their excellent service. We thank the National Institutes of Health for funding (grants HL095637, AI099404, and AR056680 to N.P.).

References

Araki Y, Mukaisyo K, Sugihara H, Fujiyama Y, Hattori T. Increased apoptosis and decreased proliferation of colonic epithelium in dextran sulfate sodium-induced colitis in mice. Oncol Rep. 2010; 24(4):869–874. [PubMed: 20811666]

Assi K, Pillai R, Gomez-Munoz A, Owen D, Salh B. The specific JNK inhibitor SP600125 targets tumour necrosis factor-alpha production and epithelial cell apoptosis in acute murine colitis. Immunology. 2006; 118(1):112–121. [PubMed: 16630028]

Babu D, Soenen SJ, Raemdonck K, Leclercq G, De Backer O, Motterlini R, Lefebvre RA. TNF-alpha/cycloheximide-induced oxidative stress and apoptosis in murine intestinal epithelial MODE-K cells. Curr Pharm Des. 2012; 18(28):4414–4425. [PubMed: 22721505]

Bavaria MN, Jin S, Ray RM, Johnson LR. The mechanism by which MEK/ERK regulates JNK and p38 activity in polyamine depleted IEC-6 cells during apoptosis. Apoptosis. 2014; 19(3):467–479. [PubMed: 24253595]

Begue B, Wajant H, Bambou JC, Dubuquoy L, Siegmund D, Beaulieu JF, Canioni D, Berrebi D, Brousse N, Desreumaux P, Schmitz J, Lentze MJ, Goulet O, Cerf-Bensussan N, Ruemmele FM. Implication of TNF-related apoptosis-inducing ligand in inflammatory intestinal epithelial lesions. Gastroenterology. 2006; 130(7):1962–1974. [PubMed: 16762619]

Bhattacharya S, Ray RM, Johnson LR. Prevention of TNF-alpha-induced apoptosis in polyamine-depleted IEC-6 cells is mediated through the activation of ERK1/2. Am J Physiol Gastrointest Liver Physiol. 2004; 286(3):G479–490. [PubMed: 14563673]

Bhattacharya S, Ray RM, Viar MJ, Johnson LR. Polyamines are required for activation of c-Jun NH2-terminal kinase and apoptosis in response to TNF-alpha in IEC-6 cells. Am J Physiol Gastrointest Liver Physiol. 2003; 285(5):G980–991. [PubMed: 12869386]

Chang L, Kamata H, Solinas G, Luo JL, Maeda S, Venuprasad K, Liu YC, Karin M. The E3 ubiquitin ligase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP(L) turnover. Cell. 2006; 124(3):601–613. [PubMed: 16469705]

Chen J, Rusnak M, Lombroso PJ, Sidhu A. Dopamine promotes striatal neuronal apoptotic death via ERK signaling cascades. Eur J Neurosci. 2009; 29(2):287–306. [PubMed: 19200235]

Chen J, Rusnak M, Luedtke RR, Sidhu A. D1 dopamine receptor mediates dopamine-induced cytotoxicity via the ERK signal cascade. J Biol Chem. 2004; 279(38):39317–39330. [PubMed: 15247297]

Chen W, Kirkbride KC, How T, Nelson CD, Mo J, Frederick JP, Wang XF, Lefkowitz RJ, Blobe GC. Beta-arrestin 2 mediates endocytosis of type III TGF-beta receptor and down-regulation of its signaling. Science. 2003; 301(5638):1394–1397. [PubMed: 12958365]

Conner DA, Mathier MA, Mortensen RM, Christe M, Vatner SF, Seidman CE, Seidman JG. beta-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to beta-adrenergic stimulation. Circ Res. 1997; 81(6):1021–1026. [PubMed: 9400383]

Emery AC, Pshenichkin S, Takoudjou GR, Grajkowska E, Wolfe BB, Wroblewski JT. The protective signaling of metabotropic glutamate receptor 1 Is mediated by sustained, beta-arrestin-1-dependent ERK phosphorylation. J Biol Chem. 2010; 285(34):26041–26048. [PubMed: 20566651]

Esposito G, Perrino C, Cannavo A, Schiattarella GG, Borgia F, Sannino A, Pironti G, Gargiulo G, Di Serafino L, Franzone A, Scudiero L, Grieco P, Indolfi C, Chiariello M. EGFR trans-activation by urotensin II receptor is mediated by beta-arrestin recruitment and confers cardioprotection in pressure overload-induced cardiac hypertrophy. Basic Res Cardiol. 2011; 106(4):577–589. [PubMed: 21369867]

Fan H, Bitto A, Zingarelli B, Luttrell LM, Borg K, Halushka PV, Cook JA. Beta-arrestin 2 negatively regulates sepsis-induced inflammation. Immunology. 2010; 130(3):344–351. [PubMed: 20465566]

Fan H, Luttrell LM, Tempel GE, Senn JJ, Halushka PV, Cook JA. Beta-arrestins 1 and 2 differentially regulate LPS-induced signaling and pro-inflammatory gene expression. Mol Immunol. 2007; 44(12):3092–3099. [PubMed: 17418896]

Goretsky T, Dirisina R, Sinh P, Mittal N, Managlia E, Williams DB, Posca D, Ryu H, Katzman RB, Barrett TA. p53 mediates TNF-induced epithelial cell apoptosis in IBD. Am J Pathol. 2012; 181(4):1306–1315. [PubMed: 22863952]

Hung CC, Ichimura T, Stevens JL, Bonventre JV. Protection of renal epithelial cells against oxidative injury by endoplasmic reticulum stress preconditioning is mediated by ERK1/2 activation. J Biol Chem. 2003; 278(31):29317–29326. [PubMed: 12738790]

Hupfeld CJ, Olefsky JM. Regulation of receptor tyrosine kinase signaling by GRKs and beta-arrestins. Annu Rev Physiol. 2007; 69:561–577. [PubMed: 17002595]

Husain KD, Coopersmith CM. Role of intestinal epithelial apoptosis in survival. Curr Opin Crit Care. 2003; 9(2):159–163. [PubMed: 12657980]

Jin S, Ray RM, Johnson LR. Rac1 mediates intestinal epithelial cell apoptosis via JNK. Am J Physiol Gastrointest Liver Physiol. 2006; 291(6):G1137–1147. [PubMed: 16798728]

Jin S, Ray RM, Johnson LR. TNF-alpha/cycloheximide-induced apoptosis in intestinal epithelial cells requires Rac1-regulated reactive oxygen species. Am J Physiol Gastrointest Liver Physiol. 2008; 294(4):G928–937. [PubMed: 18218673]

Kawamata Y, Imamura T, Babendure JL, Lu JC, Yoshizaki T, Olefsky JM. Tumor necrosis factor receptor-1 can function through a G alpha q/11-beta-arrestin-1 signaling complex. The Journal of biological chemistry. 2007; 282(39):28549–28556. [PubMed: 17664271]

Kim J, Ahn S, Rajagopal K, Lefkowitz RJ. Independent beta-arrestin2 and Gq/protein kinase Czeta pathways for ERK stimulated by angiotensin type 1A receptors in vascular smooth muscle cells converge on transactivation of the epidermal growth factor receptor. J Biol Chem. 2009; 284(18): 11953–11962. [PubMed: 19254952]

Kim M, Ashida H, Ogawa M, Yoshikawa Y, Mimuro H, Sasakawa C. Bacterial interactions with the host epithelium. Cell Host Microbe. 2010; 8(1):20–35. [PubMed: 20638639]

Kommaddi RP, Shenoy SK. Arrestins and protein ubiquitination. Prog Mol Biol Transl Sci. 2013; 118:175–204. [PubMed: 23764054]

Kook S, Zhan X, Cleghorn WM, Benovic JL, Gurevich VV, Gurevich EV. Caspase-cleaved arrestin-2 and BID cooperatively facilitate cytochrome C release and cell death. Cell Death Differ. 2014; 21(1):172–184. [PubMed: 24141717]

Kovacs JJ, Hara MR, Davenport CL, Kim J, Lefkowitz RJ. Arrestin development: emerging roles for beta-arrestins in developmental signaling pathways. Dev Cell. 2009; 17(4):443–458. [PubMed: 19853559]

Kreuz S, Siegmund D, Scheurich P, Wajant H. NF-kappaB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. Mol Cell Biol. 2001; 21(12):3964–3973. [PubMed: 11359904]

Lee T, Lee E, Irwin R, Lucas PC, McCabe LR, Parameswaran N. beta-Arrestin-1 deficiency protects mice from experimental colitis. The American journal of pathology. 2013; 182(4):1114–1123. [PubMed: 23395087]

Li H, Sun X, LeSage G, Zhang Y, Liang Z, Chen J, Hanley G, He L, Sun S, Yin D. beta-arrestin 2 regulates Toll-like receptor 4-mediated apoptotic signalling through glycogen synthase kinase-3beta. Immunology. 2010; 130(4):556–563. [PubMed: 20497256]

Li J, Wei B, Guo A, Liu C, Huang S, Du F, Fan W, Bao C, Pei G. Deficiency of beta-arrestin1 ameliorates collagen-induced arthritis with impaired TH17 cell differentiation. Proc Natl Acad Sci U S A. 2013; 110(18):7395–7400. [PubMed: 23589893]

Li P, Cook JA, Gilkeson GS, Luttrell LM, Wang L, Borg KT, Halushka PV, Fan H. Increased expression of beta-arrestin 1 and 2 in murine models of rheumatoid arthritis: isoform specific regulation of inflammation. Mol Immunol. 2011; 49(1–2):64–74. [PubMed: 21855149]

Luttrell LM, Gesty-Palmer D. Beyond desensitization: physiological relevance of arrestin-dependent signaling. Pharmacol Rev. 2010; 62(2):305–330. [PubMed: 20427692]

Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 2011; 474(7351):298–306. [PubMed: 21677746]

Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007; 129(7):1261–1274. [PubMed: 17604717]

Marjoram L, Alvers A, Deerhake ME, Bagwell J, Mankiewicz J, Cocchiaro JL, Beerman RW, Willer J, Sumigray KD, Katsanis N, Tobin DM, Rawls JF, Goll MG, Bagnat M. Epigenetic control of intestinal barrier function and inflammation in zebrafish. Proc Natl Acad Sci U S A. 2015; 112(9): 2770–2775. [PubMed: 25730872]

Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 1990; 98(3):694–702. [PubMed: 1688816]

Ostanin DV, Bao J, Koboziev I, Gray L, Robinson-Jackson SA, Kosloski-Davidson M, Price VH, Grisham MB. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. Am J Physiol Gastrointest Liver Physiol. 2009; 296(2):G135–146. [PubMed: 19033538]

Patial S, Saini Y, Parvataneni S, Appledorn DM, Dorn GW 2nd, Lapres JJ, Amalfitano A, Senagore P, Parameswaran N. Myeloid-specific GPCR kinase-2 negatively regulates NF-kappaB1p105-ERK pathway and limits endotoxemic shock in mice. J Cell Physiol. 2011; 226(3):627–637. [PubMed: 20717897]

Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol. 2014; 14(3):141–153. [PubMed: 24566914]

Porter KJ, Gonipeta B, Parvataneni S, Appledorn DM, Patial S, Sharma D, Gangur V, Amalfitano A, Parameswaran N. Regulation of lipopolysaccharide-induced inflammatory response and endotoxemia by beta-arrestins. J Cell Physiol. 2010; 225(2):406–416. [PubMed: 20589830]

Quoyer J, Longuet C, Broca C, Linck N, Costes S, Varin E, Bockaert J, Bertrand G, Dalle S. GLP-1 mediates antiapoptotic effect by phosphorylating Bad through a beta-arrestin 1-mediated ERK1/2 activation in pancreatic beta-cells. The Journal of biological chemistry. 2010; 285(3):1989–2002. [PubMed: 19915011]

Ramachandran A, Madesh M, Balasubramanian KA. Apoptosis in the intestinal epithelium: its relevance in normal and pathophysiological conditions. J Gastroenterol Hepatol. 2000; 15(2):109–120. [PubMed: 10735533]

Ray RM, Bhattacharya S, Johnson LR. Protein phosphatase 2A regulates apoptosis in intestinal epithelial cells. J Biol Chem. 2005; 280(35):31091–31100. [PubMed: 15994315]

Reiter E, Ahn S, Shukla AK, Lefkowitz RJ. Molecular mechanism of beta-arrestin-biased agonism at seven-transmembrane receptors. Annu Rev Pharmacol Toxicol. 2012; 52:179–197. [PubMed: 21942629]

Revankar CM, Vines CM, Cimino DF, Prossnitz ER. Arrestins block G protein-coupled receptor-mediated apoptosis. The Journal of biological chemistry. 2004; 279(23):24578–24584. [PubMed: 15051714]

Salaun C, Leroy C, Rousseau A, Boitez V, Beck L, Friedlander G. Identification of a novel transport-independent function of PiT1/SLC20A1 in the regulation of TNF-induced apoptosis. J Biol Chem. 2010; 285(45):34408–34418. [PubMed: 20817733]

Seidelin JB, Coskun M, Vainer B, Riis L, Soendergaard C, Nielsen OH. ERK controls epithelial cell death receptor signalling and cellular FLICE-like inhibitory protein (c-FLIP) in ulcerative colitis. J Mol Med (Berl). 2013; 91(7):839–849. [PubMed: 23371318]

Sharma D, Malik A, Lee E, Britton RA, Parameswaran N. Gene dosage-dependent negative regulatory role of beta-arrestin-2 in polymicrobial infection-induced inflammation. Infect Immun. 2013; 81(8):3035–3044. [PubMed: 23753627]

Sharma D, Malik A, Steury MD, Lucas PC, Parameswaran N. Protective Role of beta-arrestin2 in Colitis Through Modulation of T-cell Activation. Inflamm Bowel Dis. 2015

Sharma D, Packiriswamy N, Malik A, Lucas PC, Parameswaran N. Nonhematopoietic beta-Arrestin-1 inhibits inflammation in a murine model of polymicrobial sepsis. Am J Pathol. 2014; 184(8): 2297–2309. [PubMed: 24946011]

Shi Y, Feng Y, Kang J, Liu C, Li Z, Li D, Cao W, Qiu J, Guo Z, Bi E, Zang L, Lu C, Zhang JZ, Pei G. Critical regulation of CD4+ T cell survival and autoimmunity by beta-arrestin 1. Nat Immunol. 2007; 8(8):817–824. [PubMed: 17618287]

Tsutsui S, Vergote D, Shariat N, Warren K, Ferguson SS, Power C. Glucocorticoids regulate innate immunity in a model of multiple sclerosis: reciprocal interactions between the A1 adenosine receptor and beta-arrestin-1 in monocytoid cells. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2008; 22(3):786–796. [PubMed: 17965263]

Wang L, Du F, Wang X. TNF-alpha induces two distinct caspase-8 activation pathways. Cell. 2008; 133(4):693–703. [PubMed: 18485876]

Wang Y, Tang Y, Teng L, Wu Y, Zhao X, Pei G. Association of beta-arrestin and TRAF6 negatively regulates Toll-like receptor-interleukin 1 receptor signaling. Nat Immunol. 2006; 7(2):139–147. [PubMed: 16378096]

Whalen EJ, Rajagopal S, Lefkowitz RJ. Therapeutic potential of beta-arrestin- and G protein-biased agonists. Trends Mol Med. 2011; 17(3):126–139. [PubMed: 21183406]

Xu C, Reichert EC, Nakano T, Lohse M, Gardner AA, Revelo MP, Topham MK, Stafforini DM. Deficiency of phospholipase A2 group 7 decreases intestinal polyposis and colon tumorigenesis in Apc(Min/+) mice. Cancer Res. 2013; 73(9):2806–2816. [PubMed: 23361301]

Yan F, John SK, Polk DB. Kinase suppressor of Ras determines survival of intestinal epithelial cells exposed to tumor necrosis factor. Cancer Res. 2001; 61(24):8668–8675. [PubMed: 11751383]

Yan F, John SK, Wilson G, Jones DS, Washington MK, Polk DB. Kinase suppressor of Ras-1 protects intestinal epithelium from cytokine-mediated apoptosis during inflammation. J Clin Invest. 2004; 114(9):1272–1280. [PubMed: 15520859]

Zeng LX, Tao J, Liu HL, Tan SW, Yang YD, Peng XJ, Liu ZH, Jiang J, Wu B. beta-Arrestin2 encourages inflammation-induced epithelial apoptosis through ER stress/PUMA in colitis. Mucosal Immunol. 2015; 8(3):683–695. [PubMed: 25354317]

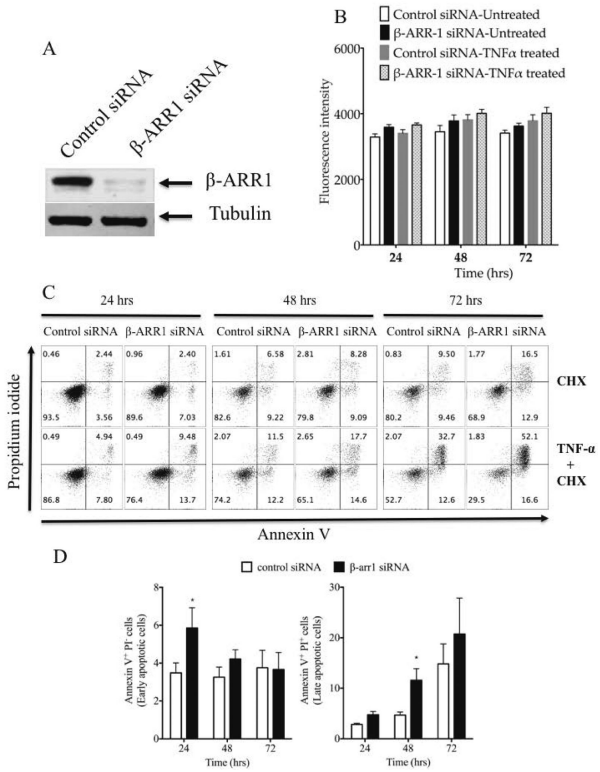

Figure 1. Effect of β-arrestin 1 knockdown on intestinal epithelial cell proliferation and apoptosis

Human intestinal epithelial cell line (SW480) was transfected with either control or β-arr1 siRNA for 48 hrs (A). Transfected cells were treated with TNF-α (20 ng/ml) at the indicated times. (B). DNA content is expressed as mean fluorescence intensity. For apoptosis, after transfection with siRNA, SW480 cells were incubated with TNF-α and CHX for the indicated time points, stained with Annexin V-FITC and propidium iodide, and analyzed by flow cytometry. The representative apoptosis pattern is shown and the apoptotic cells (Annexin V positive, PI negative) are indicated as the percentage of cells (C). Quantitative analysis of apoptosis is shown as bar graph (D). (N=5 independent experiments)

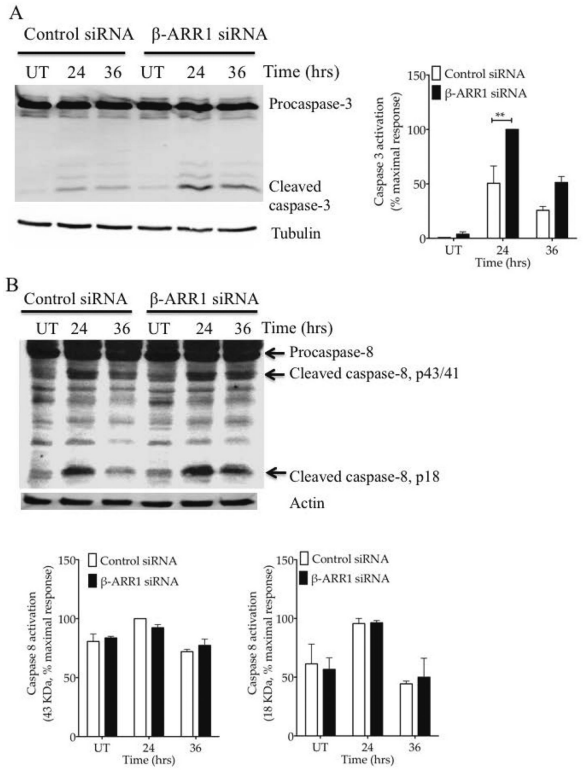

Figure 2. Effect of β-arrestin1 knockdown on TNF-α-induced caspase-3 and caspase-8 activation in intestinal epithelial cell line

Control or β-arr1 knockdown cells were treated with TNF-α and CHX for 12, 24, and 36 hours as indicated or left untreated for 36 hours (UT). Whole cell lysates were subjected to Western blotting for caspase-3 (A) and caspase-8. Representative blot is shown on the left and quantitation on the right for caspase-3. Data are mean±SEM. N=3. *p< 0.05, **P<0.001 compared with controls. Effect of the treatments on caspase-8 activation is shown in B. Representative blot is shown in the top and quantitation in the bottom for caspase-8. Data are mean±SEM. N=3.

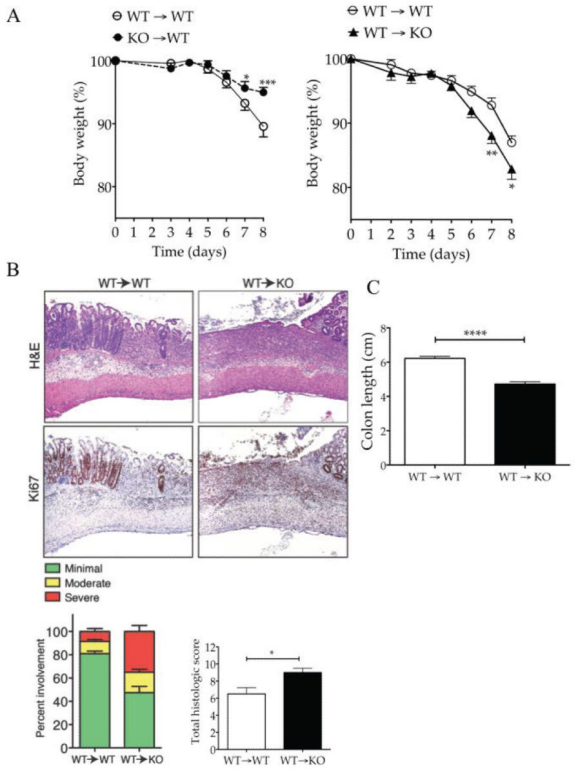

Figure 3. Increased gross and histopathological disease assessment in BM chimeric mice lacking β-arrestin1 in non-hematopoietic cells after DSS treatment

BM chimeras were subjected to 2% DSS in drinking water as described in the methods. Body weight is shown in A, histopathology is shown in B (slides on the top and quantitation in the bottom). Note that the percent mucosal involvement was compared by Fisher Exact test. P=0.021 for comparison of minimal and moderate damage between WT and non-hematopoietic KO; P<0.0001 for comparison of minimal and severe damage between WT and non-hematopoietic KO. Colon lengths are shown in C. Data are pooled from 2 independent experiments (N=10). *P<0.05, **P<0.01, ****P<0.0001 compared to the corresponding WT treatment.

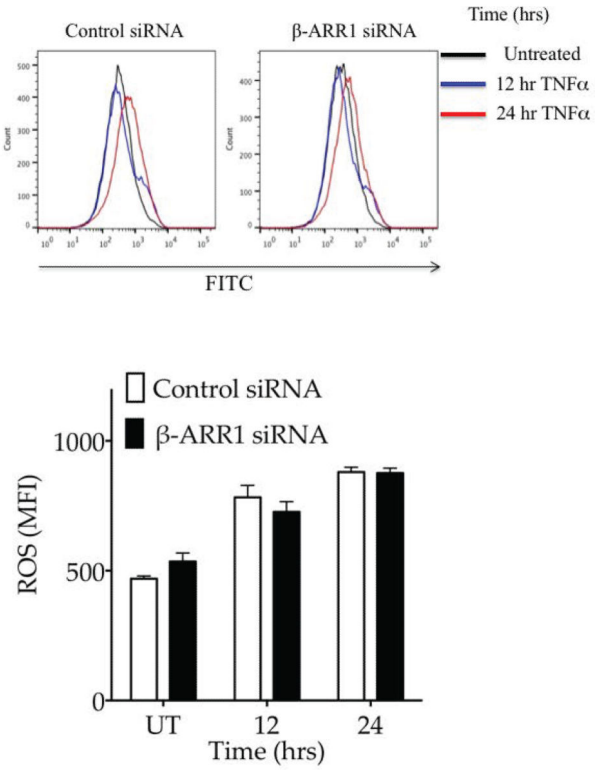

Figure 4. Effect of β-arrestin1 knockdown on TNF-α-induced reactive oxygen species in intestinal epithelial cell line

Control and β-arr1 knockdown SW480 cells were loaded with 10 μM CM-H_2DCFDA for 30 min. After washing, the cells were treated with TNF-α and CHX for 12 and 24 hours or left untreated (UT) for 24 hours. The fluorescence intensities were analyzed by flow cytometry. Untreated cells (black lines) were used as a negative control. In the top is representative histogram of one set of triplicate experiments. Blue and red lines represent 12 and 24 hours after stimulation, respectively. Quantitation of ROS production is shown as mean fluorescence intensity in the bottom (N=3 independent experiments).

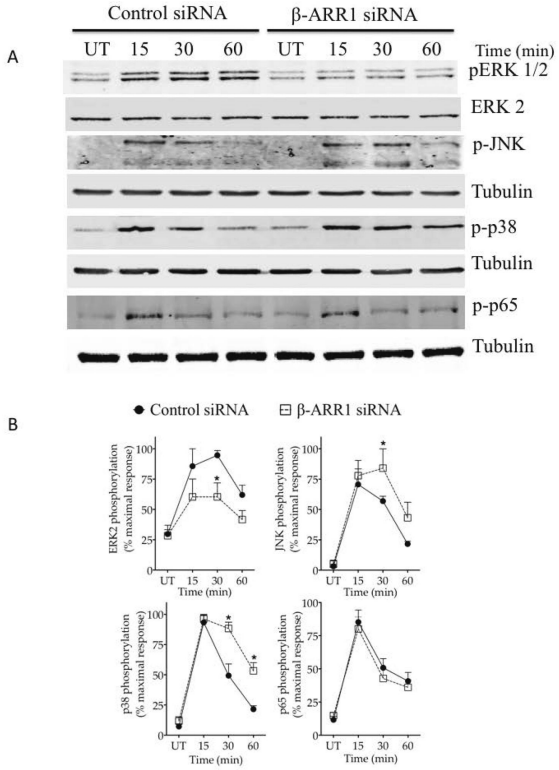

Figure 5. TNF-α-induced intestinal epithelial cell apoptosis is dependent on β-arrestin1-mediated JNK and caspase-3 signaling pathways

Control and β-arr1 knockdown SW480 cells were treated with TNF-α for 15, 30 and 60 minutes or left untreated (UT) for 60 minutes. Whole cell lysates were subjected to Western blotting for pERK1/2, pJNK, p-p38, pNFκB65. Representative blots are shown in A. Quantitation was done by normalizing phopho-blots with tubulin or ERK2 (B). N=3. *P<0.05, **P<0.01 compared with controls.