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Regulation of inflammation by β -arrestins: Not just receptor tales

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Abstract

The ubiquitously expressed, multifunctional scaffolding proteins β -arrestin1 and β -arrestin2 each affect inflammatory signaling in a variety of cell lines. In addition to binding the carboxyl-terminal tails of innumerable 7-transmembrane receptors, β -arrestins scaffold untold numbers of other plasma membrane and cytoplasmic proteins. Consequently, the effects of β -arrestins on inflammatory signaling are diverse, and context-specific. This review highlights the roles of β -arrestins in regulating canonical activation of the pro-inflammatory transcription factor NF κ B.

Keywords

Arrestins; Ubiquitination; Inflammation

β -arrestin1 and β -arrestin2 (also known as arrestin-2 and arrestin-3) were originally discovered and characterized in the context of desensitizing 7-transmembrane receptor signaling through heterotrimeric G proteins [1,2]. Subsequently, these 46–47 kDa, ubiquitously expressed proteins were found to function as multifunctional scaffolds that mediate receptor internalization [3,4] and that either diminish or activate signaling downstream of 7-transmembrane receptors, certain receptor protein tyrosine kinases [5–7], cytokine receptors [8–10], and ion channel receptors [11]. β -arrestins can interact with hundreds of proteins [12]; consequently, β -arrestins trigger or modulate myriad signal transduction pathways—including the activation of Src family tyrosine kinases [13], phosphatidylinositol-3-kinase/Akt [14], IRS-1 [15], RhoA [16,17]. Reference numbers 17 and 27 are the same NF κ B [9,10,18,19], and the mitogen-activated protein kinase families comprising ERK, JNK, and p38 isoforms (reviewed in reference [20]). β -arrestin2 promotes anti-apoptotic [7,21–23] and chemotactic [24–27] activity in a variety of cells. Through these diverse activities, in part, β -arrestins play an important role in inflammation.

Because they share 78% amino acid identity [2], it is not surprising that β -arrestin1 and β -arrestin2 associate with many of the same proteins. Indeed, ~ 50% of the proteins thus far shown to bind to *either* β -arrestin isoform bind to *both* β -arrestin isoforms (often with

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differing affinities, however [4,20,28]) [12]. This overlapping but distinct β -arrestin binding specificity manifests in two distinctive signaling patterns. First, both β -arrestin isoforms may contribute to full activation of signaling, as is the case with G protein-independent ERK1/2 signaling elicited by the β_2 -adrenergic receptor [29]. Second, one β -arrestin isoform may promote while the other β -arrestin isoform inhibits signaling, as exemplified by ERK1/2 signaling elicited by the angiotensin II AT₁ receptor [30], the protease-activated receptor-1 [31, 32], and lysophosphatidic acid receptor-1 and -2 [32]. This variability of β -arrestin-promoted ERK1/2 signaling manifests in inflammatory signaling, too.

Evidence for β -arrestin-dependent effects on inflammation has accumulated over the last 16 years—first in transfected model cell systems, and subsequently in cells and mice rendered β -arrestin-deficient genetically or by RNAi. Diverse biological systems and inflammatory stimuli have produced a multi-dimensional picture of β -arrestin-dependent regulation of inflammation.

Anti-inflammatory Roles for β -arrestins

The first use of $\betaarr2^{-/-}$ mice to assess inflammatory activity investigated lymphocyte migration triggered by CXCL12 through the 7TMR CXCR4 [24]. Comparison of $\betaarr2^{-/-}$ with cognate WT lymphocytes demonstrated that β -arrestin2 desensitized CXCR4-induced G protein-mediated signaling. However, $\betaarr2$ deficiency substantially diminished CXCL12-induced (CXCR4-dependent) migration of T and B lymphocytes [24]. Because CXCR4-dependent migration constrains lymphocytes to the bone marrow and thymus compartments [33], the ability of β -arrestin2 to augment CXCL12-induced migration ~ 2-fold could be envisioned as anti-inflammatory (a possibility not yet tested in vivo).

The role of β -arrestins in inflammatory signaling has been studied most extensively in the context of events promoting the activation of the pro-inflammatory transcription factor designated NF κ B. Canonical activation of NF κ B can be triggered through a variety of cytokine receptors [34], but we will focus here on agonist-mediated TLR4 or interleukin-1 receptor dimerization (Fig. 1). This event engenders MyD88-dependent activation of the E3 ubiquitin ligase TRAF6 (TNF receptor-associated factor 6), in a process that involves TRAF6 oligomerization, auto-ubiquitination, and subsequent synthesis of K63-linked polyubiquitin chains that are either covalently or noncovalently attached to other proteins [35] (Fig. 1). These K63-linked polyubiquitin chains activate the kinase TAK1, and co-localize TAK1 with inhibitor of NF κ B ($\text{I}\kappa\text{B}$) kinase (IKK) through noncovalent interactions [35]. Consequently, TAK1 phosphorylates and thereby activates IKK β . IKK β then phosphorylates the inhibitor α of NF κ B ($\text{I}\kappa\text{B}\alpha$) and thereby triggers K48-linked polyubiquitination and proteasomal degradation of $\text{I}\kappa\text{B}\alpha$; subsequently, NF κ B p65/p50 heterodimers are de-inhibited and can activate transcription of pro-inflammatory genes [35,36]. In this signaling model, $\betaarr1$ and/or $\betaarr2$ have been shown to inhibit NF κ B activation at two levels: (i) by binding to $\text{I}\kappa\text{B}\alpha$ and preventing its degradation [18,19], and (ii) by binding to TRAF6 and thereby impeding TRAF6 oligomerization and auto-ubiquitination [9].

The association of β arr1 and β arr2 with I κ B α was first discovered by yeast two-hybrid studies and confirmed by pull-down studies with purified proteins and by co-immunoprecipitation studies in mammalian cells [18,19]. In HeLa cells, however, tumor necrosis factor (TNF)-induced activation of NF κ B increases consequent to siRNA-mediated silencing of β arr1, but not β arr2 [19]. In contrast, β arr2 silencing in HeLa cells augments TNF-induced expression of NF κ B-dependent cytokines [18]. Furthermore, in HEK293 cells, silencing of β arr2 also augments NF κ B activation triggered by TNF [18].

β arr1 and β arr2 associate with TRAF6 via the C-terminal 100 amino acids of the β arrs [9], rather than the β arr N-terminal domain that associates with I κ B α [18]. Activation of TLR4 (by LPS), the interleukin-1 β receptor, or the receptor activator of NF κ B (RANK) promotes this β arr/TRAF6 association [9], which appears to inhibit NF κ B activation—albeit in what seems to be a context-specific manner—in several cellular systems. In β arr1^{-/-}/ β arr2^{-/-} MEFs, rescue expression of β arr1, β arr2, or their corresponding C-terminal minigenes reduces LPS-induced ubiquitination of endogenous TRAF6 [9]. However, LPS-induced secretion of the NF κ B-dependent cytokine interleukin-6 (IL-6) by the same β arr1^{-/-}/ β arr2^{-/-} MEFs was restored to WT levels by rescue expression of just β arr2, and not β arr1—perhaps because of interplay between TRAF6-dependent and ERK1/2-dependent signaling [37]. Congruently, in HeLa cells, siRNA-mediated silencing of β arr1 or β arr2 increases LPS-induced TRAF6 ubiquitination [9]. NF κ B and AP-1 promoter/reporter studies in HEK293 cells revealed that NF κ B activity is diminished or augmented, respectively, by β arr overexpression or silencing [9,37]. Silencing of β arr1 in THP-1 monocytic leukemia cells increases the LPS-induced secretion of NF κ B-dependent cytokines (IL-6, IL-8), just as β arr2^{-/-} bone-marrow-derived macrophages show greater LPS-induced I κ B kinase activity than WT macrophages [9]. Contrastingly, however, β arr2^{-/-} and WT macrophages—whether bone-marrow-derived or peritoneal—can show equivalent secretion of the NF κ B-dependent cytokines TNF and IL-6 in response to LPS, even though β arr2^{-/-} splenocytes evince greater secretion of these cytokines in response to LPS [38]. Data from fibroblast-like synoviocytes suggests the possibility of β arr isoform-specific regulation of NF κ B activity: the secretion of TNF and IL-6 in response to hyaluronan increases with β arr1 overexpression and decreases with β arr2 overexpression [39].

The inhibitory effect of β arrs on TRAF6 (and thus on NF κ B activation) may reside in the ability of β arrs to prevent TRAF6 oligomerization, which is required for auto-ubiquitination: overexpression of β arr1, β arr2, or their corresponding C-terminal minigenes in HEK293 cells inhibits the association of co-transfected Flag- and HA-tagged TRAF6 constructs [9].

In the setting of acute inflammation in vivo, β arr2 deficiency appears to augment NF κ B activation and thereby predispose to systemic disorders. Whether induced by cecal ligation and puncture [38] or by intraperitoneal injection of LPS [9], the systemic inflammatory response in β arr2^{-/-} mice substantially exceeds that in WT mice: β arr2^{-/-} mice demonstrate greater plasma levels of TNF and IL-6, as well as greater death rates over the ensuing 1–5 days than WT mice [9,38]. Arthritis induced over 5–14 days by anti-collagen IgG manifests more severely in β arr2^{-/-} than WT mice [39].

Pro-inflammatory Roles for β -arrestins

That β -arrestins promote inflammatory signaling was first appreciated in polymorphonuclear leukocytes, in which β -arrestins appear to promote degranulation. In model cell systems, IL-8 promotes β arr1-mediated CXCR1 internalization, subsequent β arr1-mediated activation of the Src-family tyrosine kinase Hck, translocation of the β arr-Hck complexes to granular compartments of the PMNs, and subsequent exocytosis of PMN granules [40].

β -arrestin2 plays a powerful pro-inflammatory role in allergic asthma, as judged by results obtained with an ovalbumin-promoted model in mice [41]. Indeed, ovalbumin-primed, methacholine-triggered bronchospasm observed in WT mice does not occur at all in β arr2^{-/-} mice, even though β arr2^{-/-} mice mount ovalbumin-specific IgG and IgE responses equivalent to those in WT mice. β arr2 deficiency abolishes ovalbumin-evoked T lymphocyte and eosinophil infiltration of the lungs, and eliminates Th2 (but not Th1) cytokine production in the lungs [41]. Mechanisms responsible for the diminished pulmonary infiltration by T lymphocytes appear to involve β arr2-mediated effects on several aspects of inflammation. First, β arr2 deficiency abrogates the secretion of the Th2 chemokine CCL22 by lung macrophages, airway smooth muscle cells, bronchiolar epithelial cells and mast cells [42] in response to aerosolized ovalbumin [41]. In this context, it is worthwhile noting that CCL22 is an NF κ B-dependent gene product [43]; thus, the failure of ovalbumin to evoke pulmonary cell CCL22 secretion in β arr2^{-/-} mice may indicate that β arr2 activity augments NF κ B activity in CCL22-secreting cells (see below). Second, echoing findings obtained with CXCL12 [24], β arr2 deficiency greatly reduces T lymphocyte migration toward CCL22. Thus, β arr2 activity is pro-inflammatory in ovalbumin-primed, methacholine-triggered allergic asthma, which develops over 3 weeks of antigen challenge and involves T cells as well as pulmonary macrophages and epithelial cells. In contrast, β arr2 activity has no effect at all in acute, LPS-promoted, methacholine-induced non-allergic asthma, which involves just pulmonary macrophages, epithelial cells, and immigrating neutrophils [41].

β arr1 plays a pro-inflammatory role downstream of TNF receptor activation in model adipocytes [44]. In these cells, β arr1 associates with TNF receptor-associated factor 2 (TRAF2), an adaptor/ubiquitin E3 ligase integral to TNF receptor-1 signaling [44]. Through binding to TRAF2, β arr1 augments TNF-induced lipolysis, JNK activation, and NF κ B-dependent gene expression, as shown by studies employing siRNA-mediated β arr1 silencing [44]. Interestingly, this TRAF2/ β arr1 association does not appear to occur in HEK293 cells [9].

β arr2—but not β arr1—also appears to promote inflammatory signaling downstream of lysophosphatidic acid receptors in mouse embryo fibroblasts (MEFs). Assessed by electrophoretic mobility shift assay and by IL-6 secretion, lysophosphatidic acid-induced NF κ B activation observed in WT MEFs is virtually abrogated in both β arr1^{-/-}/ β arr2^{-/-} double knockout and β arr2^{-/-} MEFs, yet it remains intact in β arr1^{-/-} MEFs [45]. Despite this remarkable β arr2-dependency for NF κ B activation induced by lysophosphatidic acid, there is no β arr2-dependency for NF κ B activation induced by tumor necrosis factor [45]. The locus of β arr2 activity appears to be upstream of I κ B kinase activation [45], perhaps in

promoting ubiquitination of I κ B kinase- γ , or “NEMO” [46]. Rescue expression in $\betaarr1^{-/-}/\betaarr2^{-/-}$ MEFs of $\betaarr2$, but not $\betaarr1$, restores lysophosphatidic acid-induced NF κ B activation to WT levels.

In vascular inflammation, $\betaarr1$ and $\betaarr2$ appear to play reciprocal roles: $\betaarr1$ appears to be anti-inflammatory, and $\betaarr2$ pro-inflammatory, as demonstrated by endothelial denudation studies [32]. Endothelial denudation of the mouse carotid artery engenders platelet and neutrophil adhesion to the subendothelial basement membrane; subsequently, smooth muscle cells in the artery’s tunica media proliferate and migrate through the internal elastic lamina into the subendothelial tunica intima, thereby creating “neointimal hyperplasia” over a ~ 4-week time course. Thus, neointimal hyperplasia integrates inflammatory processes of leukocytes, platelets and smooth muscle cells. Whereas endothelial injury-induced neointimal hyperplasia is greater in $\betaarr1^{-/-}$ than in WT mice, it is less in $\betaarr2^{-/-}$ than in WT mice [32]. Coupled with bone marrow transplantation, these endothelial denudation studies demonstrate that neointimal hyperplasia is augmented by $\betaarr2$ activity not in bone marrow-derived cells, but in arterial smooth muscle cells. In smooth muscle cells, $\betaarr2$ activity promotes cell proliferation and diminishes apoptosis by augmenting the activation of ERK and Akt in response to diverse stimuli in vivo and to pro-inflammatory 7TMRs in vitro [32]. In contrast, $\betaarr1$ activity in smooth muscle cells accomplishes the reverse [32]. Furthermore, in smooth muscle cells $\betaarr2$ augments TLR4-dependent NF κ B activation [10].

$\betaarr2$ also produces pro-inflammatory activity in atherosclerosis, a chronic vasculitis triggered by hyperlipidemia and responsible for the vast majority of strokes and myocardial infarctions [32]. Atherosclerosis fundamentally involves endothelial cells, smooth muscle cells, and several classes of leukocytes including monocyte/macrophages, T cells, and mast cells [47,48]. Atherosclerosis develops in $Ldlr^{-/-}$ mice over 12–20 weeks, and thus represents a more chronic form of inflammation than other models discussed in this review. Compared with $Ldlr^{-/-}$ mice, $\betaarr2^{-/-}/Ldlr^{-/-}$ mice develop 40% less atherosclerosis, with 35% fewer smooth muscle cells in their atherosclerotic lesions [32]. $\betaarr2$ could theoretically exacerbate atherosclerosis, too, by decreasing endothelial barrier function [47]: $\betaarr2$ effects this process by augmenting the endocytosis of VE-cadherin, the protein that forms adherens junctions between endothelial cells [49].

Non-inflammatory Roles for β -arrestins

Neither $\betaarr1$ nor $\betaarr2$ appear to promote conventionally inflammatory processes in a bleomycin-provoked pulmonary fibrosis model. However, each βarr promotes pulmonary fibrosis, fibroblast invasiveness, and fibroblast expression of genes related to extracellular matrix production [50]. Bleomycin-induced mortality observed in WT mice does not occur at all in $\betaarr1^{-/-}$ or $\betaarr2^{-/-}$ mice—even though WT, $\betaarr1^{-/-}$ and $\betaarr2^{-/-}$ mice demonstrate equivalent populations of macrophages, neutrophils, and lymphocytes in bronchoalveolar lavage fluid after bleomycin challenge [50]. Furthermore, inflammatory signaling in lung fibroblasts appears to be βarr -independent when it is evoked by the cytokine transforming growth factor- β . Transforming growth factor- β signals, in part, via TRAF6 and TAK1 to activate I κ B kinase, and thus NF κ B [51]. In response to transforming

growth factor- β 1, WT, β arr1^{-/-} and β arr2^{-/-} lung fibroblasts expressed equivalent amounts of the NF κ B-dependent gene products [52,53] plasminogen activator inhibitor-1 and hyaluronan [50].

Reconciliation of Apparently Discordant Data

The foregoing data offer often-discordant views of β arrs and their roles in inflammatory signaling and systemic diseases: in response to LPS, bone marrow-derived macrophages from β arr2^{-/-} mice show greater I κ B kinase activity than WT macrophages [9], but they show comparable secretion of the NF κ B-dependent gene products TNF and IL-6 [38]. Whereas β arr2 reduces secretion of NF κ B-dependent gene products from fibroblast-like synoviocytes [39], it does not affect secretion of NF κ B-dependent gene products from lung fibroblasts [50]. Furthermore, in SMCs β arr2 *augments* LPS-induced I κ B degradation and inflammation-associated SMC proliferation [10,32]. β arr2 rescue experiments in β arr1^{-/-}/ β arr2^{-/-} MEFs (performed by distinct groups) show that β arr2 either (a) increases LPS-induced activation of nuclear NF κ B [45] or (b) decreases LPS-induced TRAF6 ubiquitination and I κ B α phosphorylation [9]. β arr2 exerts similarly diverse effects in vivo on multiple read-outs regulated significantly by canonical NF κ B activation [35,43,54]: β arr2 attenuates LPS-induced or septic shock, over a time course of hours to days [9,38]; however, β arr2 does not affect LPS-primed, non-allergic asthma [41] and β arr2 actually augments allergic asthma [41], arterial neointimal hyperplasia and atherosclerosis [32], which develop over many weeks. The biological dissonance of these findings becomes all the more remarkable when one considers that most investigators used the same β arr knockout mice and β arr1^{-/-}/ β arr2^{-/-} MEFs.

To reconcile these apparently paradoxical findings, one could in some cases invoke the diversity of endpoints and signaling mechanisms in play: whether or not the endpoint in question integrates multiple β arr-dependent signaling cascades, or rather one β arr-dependent and multiple β arr-independent signaling cascades; whether the endpoint in question derives from acute versus chronic inflammation; and so on.

Another approach to understanding the diversity of inflammatory effects for β arr2, at least, involves further consideration of β arr2-mediated scaffolding: β arr2 forms a ternary complex with the E3 ubiquitin ligase TRAF6 and the ubiquitin-specific protease 20 (USP20), and can thereby facilitate the deubiquitination of TRAF6 by USP20 [10]. Silencing of β arr2 or USP20 augments TRAF6 ubiquitination [9,10,55], but silencing both β arr2 and USP20 does not produce an additive effect on TRAF6 ubiquitination; therefore, β arr2 and USP20 appear to reduce TRAF6 ubiquitination through a shared mechanism [10]. USP20 deubiquitinates both TRAF6 and β arr2—and it is deubiquitinated β arr2 that augments the anti-inflammatory effects of USP20. De-ubiquitinated β arr2 links USP20 to TRAF6 more efficiently than ubiquitinated β arr2 [10]. To associate in ternary complex with USP20 and TRAF6, the molar concentration of β arr2 must be only ~ 10–20% the concentrations of USP20 and TRAF6; otherwise, binary complexes of β arr2/USP20 and β arr2/TRAF6 become the prevalent species [10]. Thus, at least part of the variability in β arr effects on canonical NF κ B activation can be attributed to cell type- and condition-specific concentrations of β arr2, USP20, and TRAF6, and the relative concentrations of ubiquitinated and de-ubiquitinated

β arr2. β arr2 could be expected to augment NF κ B activity when deubiquitination of β arr2 is relatively slow or impaired, so that β arr2 cannot serve to tether USP20 to TRAF6. Conversely, β arr2 could be expected to attenuate NF κ B activity when β arr2-mediated scaffolding of USP20 is important for negatively regulating NF κ B activation (Fig. 2).

In light of the multiple paradoxes pertaining to β arr isoform effects on inflammation, it seems fair to infer that β arrs do not serve a unidimensional—or even a consistent—role in regulating pro-inflammatory signaling. That β arrs could be targeted therapeutically for systemic inflammatory disorders seems unlikely. Nonetheless, it remains to be determined whether β arr1 or β arr2 could possibly represent anti-inflammatory therapeutic targets for particular diseases or conditions.

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Abbreviations

βarr	β -arrestin
IL	interleukin
IκBα	inhibitor α of NF κ B
IKK	I κ B kinase
MEF	mouse embryo fibroblast
LPA	lysophosphatidic acid
TLR4	Toll-like receptor-4
TNF	tumor necrosis factor
TNFR1	TNF receptor-1
TRAF	TNFR-associated factor
7TMR	7-transmembrane receptor

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Fig. 1. Model for βarr-mediated regulation of inflammatory signaling

Pictured are NFκB activation pathways downstream of the 7-transmembrane receptor for the platelet product lysophosphatidic acid (LPA-R), the TLR4, and the TNF receptor-1 (TNFR1). **LPA-R-mediated activation of NFκB** requires a βarr2-scaffolded “signalosome” comprising CAR-MA3, Bcl10, MALT1, and TRAF6, which autoubiquitinates and generates K63-linked polyubiquitin that binds to NEMO to activate IκB kinase (IKK) and thence NFκB. By binding to IκBα, however, either βarr1 or βarr2 can inhibit IκBα degradation in the 26S proteasome, and thereby inhibit NFκB activation. **TLR4 or interleukin-1 receptor dimerization** triggers phosphorylation and thus activation of IRAK1 (IL-1 receptor-associated kinase), which then associates with and activates TRAF6, whose K63 polyubiquitin chains bind to NEMO and to the TAB2 subunit of the kinase TAK1, which autophosphorylates/activates and then phosphorylates and thereby activates IKK. **TNFR1 activation** recruits TRAF2 to at least 2 signaling complexes: (a) the classical complex involving TRADD and the ubiquitin E3 ligase cIAP1, with polyubiquitin- and phosphorylation-mediated activation of IKK similar to that described above; (b) a second complex involving βarr1 (but perhaps not βarr2), Src and Gq. “UUU” denotes polyubiquitin chains involved in promoting signaling; “+” denotes stimulation; “-” denotes inhibition. Schematic adapted from references 35, 44 and 46.

Fig. 2. βarr2-mediated regulation of ubiquitin-dependent NFκB signaling

Activation of TLR4 triggers ubiquitination of βarr2 and TRAF6 {Jean-Charles, 2016 #3951}. Because ubiquitinated βarr2 fails to scaffold USP20 to TRAF6 effectively, there is less deubiquitination of TRAF6 and greater NFκB signaling when βarr2 is ubiquitinated. Upon deubiquitination by USP20, however, βarr2 avidly scaffolds USP20 to TRAF6. Consequently, USP20 deubiquitinates TRAF6 and thereby inhibits NFκB signaling. This figure was originally published in P-Y Jean-Charles et al J Biol Chem. 2016 Apr 1;291(14):7450–64.