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Interleukin-4-induced β-catenin regulates the conversion of macrophages to multinucleated giant cells

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ABSTRACT

The cytokine interleukin-4 (IL-4) exerts pleiotropic effects on macrophages as it plays a key role in the immune response to infectious agents, allergens, and vaccines. Macrophages exposed to IL-4 drastically change their gene expression and metabolic state to adjust to new functional requirements. IL-4 also induces macrophages to fuse together and form multinucleated giant cells (MGCs). MGC formation is associated with chronic inflammation resulting from persistence of pathogenic microorganisms or foreign materials in tissues. Very little is known, however, about the mechanisms regulating IL-4-induced macrophage-to-MGC conversion. We observed a dramatic increase in β-catenin protein but not mRNA amount in mouse macrophages following exposure to IL-4. To investigate the role of β-catenin in macrophages, we generated mice with a myeloid cell-specific deletion of the β-catenin gene. Ablation of β-catenin expression did not affect the viability of macrophages or impair expression of known IL-4-inducible genes. Intriguingly, β-catenin-deficient macrophages incubated with IL-4 formed MGCs with markedly greater efficiency than wild-type macrophages. Similar increases in multinucleated cell formation were detected in the peritoneal cavity of myeloid cell-specific β-catenin knockout mice injected with chitin, which is known to induce endogenous IL-4 production. Our findings reveal β-catenin as a novel regulator of macrophage responses to IL-4, and suggest that therapeutic modulation of its expression or function may help enhance the effectiveness or ameliorate the pathology of IL-4-driven immune responses.

1. Introduction

Macrophages are a heterogeneous group of myeloid cells that play a key role in immune defense and tissue homeostasis. Although present in some abundance in normal tissues, macrophages are recruited in greater numbers to inflamed body sites. Macrophages serve a wide variety of functions, ranging from phagocytic removal of invading pathogens to production of signaling molecules orchestrating inflammatory responses and tissue repair (Murray and Wynn, 2011). Further, specific subpopulations of macrophages are known to regulate metabolic processes as diverse as bone mineral resorption, iron recycling, and fatty acid catabolism (Novack and Teitelbaum, 2008; Ganz, 2009; Chawla et al., 2011). The versatility of macrophages is attributable to their phenotypic plasticity: macrophages undergo shifts in gene expression and adopt distinct functional characteristics when exposed to different cytokines. Stimulation by the cytokines interferon (IFN)-γ and interleukin (IL)-4, for instance, results in the expression of distinct phenotypes, referred to as M1 and M2, respectively (Lawrence and Natoli, 2011; Sica and Mantovani, 2012). These two macrophage fates are geared to contrasting immune defense mechanisms effective against different types of pathogenic microorganisms.

During an active immune response, IL-4 is produced by T cells, mast cells, eosinophils, and basophils (Seder et al., 1991; Voehringer et al., 2004). IL-4 functions to promote Th2 effector cell development and antibody-mediated immunity by exerting pleiotropic effects on multiple cell types (Paul and Zhu, 2010). Other cellular sources of IL-4 have recently been identified that come into play in various physiological contexts. Most notably, adipocytes and tumor cells have been suggested to produce IL-4 and thereby induce fat- and tumor-associated macrophages to differentiate into M2 phenotype (Kang et al., 2008; Gocheva et al., 2010). The precise role of M2-polarized macrophages has not been unequivocally determined, but evidence suggests a link to tissue repair, metabolic control, and tumor growth (Gordon and Martinez, 2010). IL-4 signals to reprogram gene expression in macrophages and other target

Abbreviations: AJ, adherens junctions; GM-CSF, granulocyte-macrophage colony stimulating factor; IFN, interferon; IL, interleukin; IRF, Interferon regulatory factor; KO, knockout; MGC, multinucleated giant cell; PBS, phosphate-buffered saline; qPCR, quantitative PCR; RANKL, RANK ligand.

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cells. IL-4 binding to its receptor leads to activation of the transcription factor Stat6, which is required for the expression of many IL-4-inducible genes (Goenka and Kaplan, 2011).

M2 gene expression and phenotype apart, IL-4 induces radical changes in macrophage morphology and behavior: IL-4-exposed macrophages aggregate and fuse together, forming syncytia called multinucleated giant cells (MGCs). MGCs are most frequently observed in tissues afflicted with chronic inflammation. Persistent microbial infection and foreign body implantation can create such tissue environments, and are indeed associated with MGC formation (Helming and Gordon, 2009a). IL-4-induced macrophage fusion and MGC formation likely reflect an attempt to increase the capacity of macrophages to contain and destroy invading non-self entities. Studies employing gene knockout and knockdown approaches and the use of function-blocking antibodies (Kyriakides et al., 2004; Yagi et al., 2005; Helming and Gordon, 2007; Jay et al., 2007; Moreno et al., 2007; Pajcini et al., 2008; Helming et al., 2008, 2009b; MacLauchlan et al., 2009; Van den Bossche et al., 2009; Yu et al., 2011) revealed that, in addition to the IL-4 receptor and Stat6, MGC formation depends on several other proteins in diverse functional categories: CD36, DC-STAMP, E-cadherin, and TREM-2 (cell surface interaction); CCL2 (chemotaxis); Rac1, Dock180, DAP12, and Syk (intracellular signaling); MMP9 (proteolysis); and NF-κB p105/p50 (gene transcription). Analysis of Dicer-deficient macrophages has shown that a microRNA-based mechanism is at work to hold MGC formation in check (Sissons et al., 2012). However, few proteins have been documented to serve as negative regulators of IL-4-induced MGC formation.

Here we discover that β-catenin functions to inhibit the conversion of IL-4-exposed macrophages to MGCs. By generating and investigating mice with a deletion of the β-catenin gene, *Ctnnb1*, in myeloid cells, we find that ablation of β-catenin expression in macrophages leads to marked increases in the efficiency of the formation of MGCs and multinucleated cells of smaller size in vitro and in vivo. Our study provides new insight into how IL-4 signaling and macrophage fusion are regulated, and identify a new role for β-catenin in macrophage biology.

2. Materials and methods

2.1. Animals

C57BL/6J mice were used to isolate bone marrow and prepare bone marrow-derived macrophages. Mice with floxed (fl) *Ctnnb1* alleles (Brault et al., 2001) and *LysMCre* knockin mice (Clausen et al., 1999), both on a C57BL/6J background, were obtained from the Jackson Laboratory. These mice were crossed to generate myeloid cell-specific *Ctnnb1* knockout mice (*Ctnnb1*$^{fl/fl}$*-LysMCre*). All animal studies were conducted under IACUC-approved protocols.

2.2. Macrophage preparation and culture

Bone marrow isolated from the tibia and femur of mice were cultured in a differentiation medium containing high-glucose Dulbeccos' modified Eagles medium (DMEM), 10% fetal bovine serum, and 10 ng/ml macrophage-colony stimulating factor (M-CSF; PeproTech) for seven days. Plastic Petri dishes were used to facilitate recovery of trypsin- and EDTA-treated macrophages. Macrophages in tissue culture plates were treated with lipopolysaccharide (LPS; Sigma–Aldrich) and the following cytokines: granulocyte-macrophage colony stimulating factor (GM-CSF), IFN-γ, IL-4 and IL-6 (all from PeproTech); and RANK ligand (RANKL) and IL-10 (all from R&D Systems).

Table 1
Oligonucleotide primers used in real-time qPCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Arg1	caagacagggctcctttcag	ttcccaagagttgggttcac
Ccl17	caggaagttggtgagctggt	catccctggaacactccact
Ccl2	gccagctctctcttcctcca	cccagaagcatgacagggac
Cdh1	gagaacggtggtcaaagagc	tgtcccgggtatcatcatct
Chi3l3	ccagcatatgggcatacctt	gggcaccaattccagtctta
Ctnnb1	cagatgcagcgactaagcag	gctgcactagagtcccaagg
Mmp13	tttattgttgctgcccatga	ggtccttggagtgatccaga
Mrc1	agtgatggaaccccagtgac	gttctcatggcttggctctc
Ppia	atggtcaaccccaccgtgt	ttcttgctgtctttggaactttgtc
Sdc4	atctggatgacacggaggag	gcattctcagggatgtggtt

2.3. Protein and RNA analysis

Whole cell lysates were prepared as described (Park et al., 2004) and analyzed by immunoblot using antibodies against the following proteins: arginase-1 (sc-18354), iNOS (sc-651), IRF4 (sc-6059), IRF8/ICSBP (sc-6058; all from Santa Cruz Biotechnology); β-catenin (610153; BD Biosciences); IRF5 (10547-1-AP; Proteintech); E-cadherin (3195; Cell Signaling Technology); Ym-1 (01404; Stemcell); and actin (A4700; Sigma–Aldrich). Total RNA was extracted from cultured macrophages using Trizol (Life Technologies). cDNA was synthesized from total RNA using a SuperScript II cDNA synthesis kit (Life Technologies). Real-time quantitative PCR (qPCR) was performed using Fast SYBR Green master mix (Life Technologies). The oligonucleotide primers used in real-time qPCR are listed in Table 1.

2.4. MGC formation in vitro

Macrophages were incubated in Permanox chamber slides (Lab-Tek) at 2×10^6/ml for 16 h, treated with IL-4 (25 ng/ml), and further incubated for 7 d. Cells on the slide were stained with 0.09% crystal violet and analyzed by bright-field microscopy.

2.5. Immunofluorescence microscopy

Cells fixed with 4% formaldehyde and permeabilized with cold 0.5% Triton X-100 were incubated with phalloidin-tetramethylrhodamin B isothiocyanate (Sigma–Aldrich) and DAPI (Invitrogen). After treatment with the anti-fading agent VectaShield (Vector Laboratories), the signal was visualized using an A1R confocal microscope system with an ×60 oil-immersion lens (Nikon).

2.6. Chitin-induced peritonitis

Chitin (Sigma–Aldrich) was suspended in phosphate-buffered saline (PBS), filtered through a 70-μm strainer, centrifuged, and resuspended in ten times the packed pellet volume of PBS. Mice were injected intraperitoneally with 0.2 ml of 10% chitin suspension. Peritoneal lavage was collected 6 d after chitin injection, and the cells were mounted on slides by Cytospin centrifugation, stained with Diff-Quik solutions (Siemens Healthcare Diagnostics), and analyzed by bright-field microscopy.

3. Results

3.1. IL-4 induces a drastic increase in β-catenin protein but not mRNA amount in macrophages

Growing evidence suggests a role for β-catenin in macrophage biology (Otero et al., 2009; Yang et al., 2010). We sought to determine the role of β-catenin in cytokine-induced changes in