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Molecular Characterization of Macrophage-Biomaterial Interactions

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Abstract

Implantation of biomaterials in vascularized tissues elicits the sequential engagement of molecular and cellular elements that constitute the foreign body response. Initial events include the non-specific adsorption of proteins to the biomaterial surface that render it adhesive for cells such as neutrophils and macrophages. The latter undergo unique activation and in some cases undergo cell-cell fusion to form foreign body giant cells that contribute to implant damage and fibrotic encapsulation. In this review, we discuss the molecular events that contribute to macrophage activation and fusion with a focus on the role of the inflammasome, signaling pathways such as JAK/STAT and NF- κ B, and the putative involvement of micro RNAs in the regulation of these processes.

Keywords

Biomaterials; Foreign body response; Foreign body giant cells; Inflammation; Inflammasome; Macrophage

7.1 Introduction

The foreign body response (FBR) is initiated following injury due to biomaterial implantation. An influx of proteins from the blood and interstitial fluid creates a random and temporary proteinaceous coating on the biomaterial surface. Proteins from serum as well as interstitial proteins attach and undergo changes in structure including denaturation on the surface of the implant and aid in subsequent cell adhesion [1–3]. Additionally, platelets release chemoattractant signals that stimulate cell migration and fibrinogen that contributes to the formation of a provisional matrix [2]. Following protein interactions, inflammatory cells such as neutrophils and monocytes migrate to the implant, interact with surface-adsorbed proteins and undergo activation. Recruited macrophages, as well as resident

macrophages, are induced to secrete their own chemoattractant signals and cytokines, which contribute to the development of the FBR [1]. Recent evidence has also implicated activation of the inflammasome in this process, which leads to the production of pro-inflammatory cytokines such IL-1 β , and eventual fibrotic encapsulation of the implant. In contrast to the typical wound environment, macrophages undergo unique activation and a subset undergoes fusion to create foreign body giant cells (FBGC) [4]. FBGCs are considered a hallmark of the FBR and can cause direct degradation of the implanted biomaterial, which often leads to its malfunction [1, 4, 5]. Specifically, it has been shown that FBGCs secrete reactive oxygen species, degradative enzymes, and create an acidic environment at the biomaterial interface [4]. In fact, direct erosion of implants by FBGCs has been demonstrated by scanning electron microscopy [5, 6]. Subsequently, pro-fibrotic signals at the implant site induce the formation of a collagenous and largely avascular capsule, which envelopes the biomaterial within 2–4 weeks after implantation [2, 4]. Confinement in the capsule prevents true integration of the implant with the surrounding tissue, which is responsible for the loss of function for numerous biomaterials including sensors [1, 7, 8]. Figure 7.1 provides an overview of the participation of macrophages in the FBR.

7.2 Biomaterials and the Inflammasome

Various stimuli including silica, uric acid, ATP, and disruption of cellular integrity induce the assembly of a protein complex consisting of nucleotide-binding domain and leucine-rich repeat-containing –type (Nlrp), apoptosis-associated speck-like protein containing CARD (Asc), and Caspase-1, known as the inflammasome [9,10]. Caspase-1 is normally inactive but undergoes activation during complex formation. The primary role of the inflammasome is to convert pro- IL-1 β to IL-1 β , which is then secreted into the extracellular milieu [10–12]. Induction of pro- IL-1 β production is mediated by a separate signal involving Toll-like receptor (TLR) 4. Therefore, engagement of TLR4 and Nlrp leads to the coordinated production, activation, and secretion of IL-1 β . The stimuli mentioned above can induce inflammasome formation and caspase-1 activation by causing K⁺ efflux, generation of reactive oxygen species, or lysosomal destabilization. Macrophage-biomaterial interactions have been shown to induce IL-1 β release in a number of in vitro and in vivo studies suggesting the involvement of the inflammasome. However, studies demonstrating a direct link between macrophage-biomaterial interactions and inflammasome activation are limited. St Pierre et al., showed in a series of in vitro studies that uptake of titanium microparticles by macrophages induced the release of IL-1 β and utilized siRNAs against inflammasome components to inhibit this process [13]. Similarly, Maitra et al showed that isolated polyethylene-based implant-derived particles and alkane polymers could induce pro- IL-1 β production and IL-1 β release in macrophages [14]. In this study, inhibition of inflammasome components was not pursued but the authors did demonstrate caspase-1 activation. In addition, numerous studies have shown that nanoparticles and microparticles can activate the inflammasome. More recently, Bueter et al demonstrated activation of the inflammasome by chitosan, which based on studies with selective inhibitors, was dependent on K⁺ efflux, reactive oxygen species, and lysosomal destabilization [15]. This and the studies described above involve biomaterial-based foreign bodies that are readily taken up

by single cells. However, many in vitro studies show release of IL-1 β by macrophages in contact with biomaterials suggesting the involvement of mechanisms that do not involve uptake. Consistent with this suggestion, investigators showed that the inflammasome is activated in the context of cell-biomaterial interactions [16]. Specifically, addition of 150 μ m poly-methyl methacrylate (PMMA) microspheres to macrophages induced inflammasome activation and IL-1 β secretion. Similarly, injection of the same microspheres in a mouse intraperitoneal model resulted in increased levels of IL-1 β . Utilizing the same model, investigators showed lack of IL-1 β production in mice deficient for Caspase-1, Nlrp3, or Asc. Interestingly, in long term subcutaneous (SC) implant studies (4 weeks), mice deficient in either Caspase-1 or Asc displayed reduced implant encapsulation. In contrast, encapsulation was normal in Nlrp3 KO mice indicating the participation of a separate Nlrp receptor in the progression of the FBR. Therefore, more research is needed to identify the inflammasome components that mediate implant fibrosis. Figure 7.2 illustrates putative modes of inflammasome activation by biomaterials.

7.3 Macrophages and FBGCs in the FBR

Homologous cell fusion is a highly orchestrated process that occurs in numerous cell types under both physiological and pathological conditions, including trophoblasts in placental development, myoblasts in skeletal muscle formation, and cells of the monocytic lineage in osteoclast and FBGC formation [17–21]. The latter can be induced in vitro by treatment of macrophages plated on fusion-permissive surfaces, such as naked polystyrene, with IL-4. In vitro fusion studies involving macrophages from IL-4R α knockout mice have demonstrated the importance of IL-4 in the fusion process [22]. Interestingly, FBGC formation was recently shown to be normal in IL-4R α knockout mice suggesting the existence of additional fusogenic signals in vivo [23]. FBGCs can be damaging to biomaterials and devices and contribute to inflammation at the implant site [1, 4, 19, 21, 24, 25]. In addition, macrophages and FBGCs provide signals that contribute to the formation of the collagenous capsule. For example, they secrete pro-migratory molecules and TGF- β , which leads to recruitment of fibroblasts that deposit extracellular matrix and encapsulate the implant [1, 4, 26]. Foreign body capsules can reach thickness of 50–200 μ m and completely envelope implants in a largely avascular space that consists of dense and highly organized collagen fibers [1, 26].

7.4 Macrophage Activation in the FBR

Macrophages have been recently categorized based on the expression of specific molecules that reflect their activation state [27]. Most commonly, they are subdivided into two activation/polarization states: classically activated (M1) or alternatively activated (M2) but these states should be considered as broad characterizations. M1 macrophages are thought to be involved in pro-inflammatory signaling whereas M2 are classified as anti-inflammatory cells that contribute to tissue repair [27–29]. Both of these states can be induced in vitro by treatment of cells with IFN- γ /LPS (M1) or IL-4 (M2). Because FBGC formation can be induced by IL-4 these cells often categorized as M2 [29–31]. However, recent studies support the idea of a M1/M2 activation continuum rather than distinct states [27, 29, 32–35]. In fact, in vivo analysis of traditional M1 and M2 activation markers in an interperitoneal (IP) implantation model by qRT-PCR, immunohistochemistry, and enzyme linked

immunosorbent assays (ELISAs) demonstrated a unique polarization state that was highlighted by both M1 and M2 markers [36]. Specifically, FBGC expressed both M1 (iNOS, IL-1 β , TNF) and M2 (Arg1, CD36, IL-10) markers [36]. Likewise, analysis of gene expression following IP implantation of boiled egg white demonstrated induction of both M1 (TNF, IL-6) and M2 (IL-4, IL-10) markers during the ensuing FBR [32]. Finally, analysis of subcutaneous polyvinyl alcohol (PVA) sponge implants demonstrated overlapping M1-M2 macrophage phenotypes during the FBR, with cells expressing TNF, IL-6, Arg1, TGF β , and Ym1 [33]. These studies highlight the unique plasticity and activation state of macrophages during the FBR [29, 34].

7.5 Molecular Pathways

7.5.1 JAK/STAT Pathway

Fusion of macrophages is the consequence of a multistep mechanism induced by IL-4 and followed by the acquisition of a fusion competent state, chemotaxis, and subsequent cytoskeletal rearrangements during and after fusion [17, 19, 37, 38]. Progression to fusion results in the increased expression of cell surface and secreted molecules including DNAX-activating protein of molecular mass 12 kDa (DAP12), dendritic cell-specific transmembrane protein (DC-STAMP), matrix metalloproteinase 9 (MMP9), monocyte chemotactic protein-1 (MCP-1), and epithelial-cadherin (ECad) [2, 4, 17, 39, 40]. As mentioned above, induction of a fusion-competent state in vitro can be achieved by addition of IL-4 and involves at least two separate pathways including JAK/STAT [37]. As shown in Fig. 7.3, IL-4 induces the JAK1/3 and STAT6 signaling cascade, leading to upregulation of ECad and β -catenin that localize to the cell periphery where they are thought to facilitate cell-cell interactions [37, 41].

7.5.2 MCP1 and Rac1-Dependent Cytoskeletal Remodeling

IL-4 induces DAP12-dependent signaling through the ITAM motif and TREM2 receptor, with downstream SYK signaling increasing DC-STAMP expression [42]. Moreover, machinery reminiscent of macrophage phagocytosis, including MCP-1 mediated Rac-1-dependent cytoskeleton rearrangements and phosphatidyl serine (PtdSer) exposure and subsequent recognition by CD36, have also been linked to macrophage fusion [17, 43–45]. The reason the two pathways are considered separate involves observations in MCP1-KO mice and their macrophages that are defective in fusion but display normal upregulation of ECad and β -catenin in response to IL-4. In contrast, they display reduced Rac-1 activation and Rac-1-mediated cytoskeletal remodeling as well as TNF and MMP-9 expression [2, 37]. However, fusion in MCP-1 macrophages can be restored by addition of exogenous MMP-9, which leads to changes in the subcellular localization of E-cad from the cell periphery to the cytoplasm. These observations suggest that the secretion of MMP-9 and the possible cleavage of ECad represent a point where the two pathways display functional overlap [37]. While several molecular mediators of fusion have been implicated in the FBR, the exact signaling pathways regulating this process remain unknown and it is anticipated that their identification would lead to the development of novel strategies to attenuate FBGC formation and the FBR [4].

7.5.3 TNF and NF- κ B Pathway

In vitro and in vivo studies have demonstrated the importance of TNF in the FBR [4, 32, 33, 37]. In fact, analysis of levels of TNF during macrophage-biomaterial interactions can be helpful in evaluating the biocompatibility of new materials [4]. For example, TNF has been used as a marker of inflammation and indicator of severity of the FBR in studies ranging from the effect of topographical alterations, lymphocyte enhancement of FBGC activation, and the biocompatibility of novel materials such as poly(ethyleneglycol)-based hydrogels [46–48]. The importance of TNF is apparent when one considers the fact that the fusion-deficient phenotype of MCP-1 macrophages can be rescued via exogenous TNF treatment [37]. TNF induction in macrophages during the FBR is intriguing because it is a potent inducer of the NF- κ B pathway. In the absence of activation, the canonical NF- κ B components p50 and p65 (RelA) are held in the cytoplasm by the inhibitory I κ B. When present, TNF induces activation of the IKK complex, promoting the phosphorylation and ubiquitination of I κ B, which is subsequently degraded allowing the release and translocation of the p50-p65 heterodimer complexes to the nucleus where they induce transcription of target genes (Fig. 7.3) [49]. Alternatively, the non-canonical NF- κ B pathway involves NIK-dependent induction of IKK which phosphorylates the p100 precursor thereby releasing the p52-RelB complex [49]. Canonical activation of NF- κ B has been noted *in vivo* following implantation of titanium and copper implants in rats and propylene mesh in mice [50, 51]. Additionally, induction of the non-canonical NF- κ B pathway has been demonstrated as essential to RANKL mediated osteoclast fusion [52]. Although the canonical NF- κ B pathway has been shown to be important in IL-4-induced macrophage fusion and the non-canonical pathway for osteoclast fusion, it has been suggested that cross talk between pathways does occur, potentially allowing for compensation [53]. Nevertheless, It has been recently established that the canonical NF- κ B pathway is required for macrophage fusion during the FBR both in vitro and in vivo [36]. Specifically, induction and nuclear translocation of NF- κ B components p50 and RelA were shown at day 3 following IL-4 stimulation. NF- κ B induction occurred in temporal manner consistent with TNF expression and was minimal in fusion-deficient MCP-1 KO mice. Additionally, inhibition of canonical NF- κ B pathway by treatment with the pharmacological inhibitor Bay11, resulted in decreased fusion. More importantly, induction and nuclear translocation of p50/RelA was observed in vivo in implant-adherent macrophages undergoing fusion at day 4 following implantation in an IP model [36]. These observations suggest that TNF contributes to FBGC formation and the FBR, in part, by activating the canonical NF- κ B pathway. However, the downstream effects of this pathway and the genes that are regulated by p50/p65 in this process have not been identified.

7.6 FBGC Formation and FBR Phenotypes in Genetically Modified Mice

With the advent of genetically modified mice, investigators have utilized models of biomaterial implantation in order to elucidate the contribution of specific molecules in the FBR. Despite the lack of standardized approaches in these studies and the variable approaches used, such as multiple implantation locations and time points, numerous biomaterials, and different modes of analysis, the cumulative body of acquired knowledge is informative. For example, it was shown in short term studies that mice deficient in either

plasminogen or fibrinogen displayed reduction in cell recruitment and/or cell attachment to biomaterials [54]. In addition, mice lacking components critical for monocyte/macrophage recruitment such as E- and P-selectin displayed reduced accumulation of inflammatory cells in an IP implantation model and this was associated with a reduced fibrotic response [55]. Similarly, mice lacking MCP-1 displayed reduced macrophage accumulation and FBGC formation and significant attenuation of capsule thickness in an IP implant model [37]. Interestingly, the same mice with SC implants displayed reduced FBGC formation despite normal macrophage recruitment and capsule thickness [2]. Several knockout mice or cells isolated from them displayed altered FBGC formation including MMP-9, DC-STAMP, DAP12, IL-4R α , MT1-MMP, plasma fibronectin, osteopontin, PTPN12, STAT6, and CD36 [22, 40, 42, 44, 56–61]. As mentioned in Section 2 above, Helming et al demonstrated compromised fusion of IL4R α -KO macrophages in vitro [22]. Consistent with the findings of Helming et al, anti-IL-4 antibodies were shown to block FBGC formation in a cage implant model [62]. In contrast, Yang et al showed normal FBGC formation in IL4R α -KO mice in a SC implant model [23]. Therefore, the requirement for IL-4 signaling and perhaps other signals in FBGC formation in vitro and in vivo remains to be elucidated. Moreover, the complex phenotype of biomaterial-adherent macrophages, featuring characteristics of both M1 and M2 activation, suggests the contribution of additional signaling molecules.

Alterations in capsule formation have also been detected in genetically modified mice, including those lacking the angiogenesis inhibitor thrombospondin-2 (TSP2), which formed capsules with increased vessel density and aberrant collagen fibers [63]. SPARC-KO mice displayed reduced collagen capsule thickness, and double deletion of SPARC and its homologue have resulted in increased vessel density [64,65]. Plasminogen activator inhibitor-1 KO mice displayed reduced fibrosis in a PVA sponge implant model [66]. More recently, Zaveri et al demonstrated a surprising role for macrophage integrin Mac1 in influencing capsule thickness [67]. Obviously, these molecules represent significant variation in function (enzymes, receptors, cell adhesion proteins, cytokines, extracellular matrix proteins) and subcellular localization (cytoplasmic, membrane-bound, secreted), which highlights the complexity of the processes they regulate. Moreover, several of these molecules have been shown to be induced significantly during FBGC formation and progression of the FBR suggesting that regulation of gene expression plays a significant role in these processes.

7.7 MicroRNAs and FBGC Formation

MicroRNAs (miRs) are small noncoding RNAs that regulate gene expression via post-transcriptional modification of transcripts [68]. See Eulalio et al and Krol et al for detailed reviews on the generation and mode of action of miRs [70, 71]. MiRs have confirmed participation in essentially all cellular processes examined to date, including cellular development, metabolism, apoptosis, proliferation, and differentiation [71–73]. If one considers that a single miR may influence post-transcriptional control of hundreds of targets, and that an mRNA transcript will be influenced by many miRs, it is not surprising that miRs regulate over half of the human genome [72–74]. Though little work has been done regarding the role of miRs in cellular fusion, evidence exists that they are involved in monocyte/macrophage differentiation and processes involved in cell fusion such as

cytoskeletal remodeling and the NF- κ B pathway [75–78]. In addition, miRs haven been shown to regulate several molecules that have been implicated in FBGC formation. For example, miRs regulate the hematopoietic stem cell lineage, including the differentiation of monocytes into macrophages, which strongly suggests their importance in determining macrophage phenotype [72]. Furthermore, miR-21 has recently been linked to the process of differentiation of monocyte-derived dendritic cells, a process that is dependent on IL-4 and granulocyte- macrophage colony stimulating factor (GM-CSF) [79]. MiR-705 has been shown to regulate MMP9 expression in the uterine matrix, and miRs 143/145 regulate cytoskeletal remodeling during phenotype switch in smooth muscle cells [80, 81]. More importantly, miRs have been implicated in homotypic cell fusion including that of myoblasts, osteoclasts, and most recently FBGC. Sugatini et al. reported that miR-223 and miR-21 regulated RANKL-mediated osteoclastogenesis [82, 83]. Unlike miR-223 where the targeted transcript(s) involved in the regulation of osteoclast formation are not known [84–86], miR-21 was shown to downregulate PDCD4 to promote fusion [83]. In addition, miR-7b has been demonstrated to directly target DC-STAMP during osteoclastogenesis, thus inhibiting NFATC1 and c-FOS to attenuate fusion [87]. As mentioned above, myoblast fusion during developmental myogenesis as well as following injury is also regulated by microRNAs. Specifically, reduction of myoblast fusion was observed to be regulated by miR-1192 targeting HMGB1 as well as miR-206 and miR-1 downregulating CX43 gap junctions during myogenesis [88, 89]. The participation of miRs in FBGC formation is largely unexplored with a single study showing that miR-7a-1 can regulate DC-STAMP during IL-4 induced macrophage fusion [90]. Finally, deletion of dicer, which is a central molecule in miR processing, resulted in a significant increase of IL-4 dependent fusion [90].

7.8 Conclusion

Investigation of the molecules and signaling pathways that regulate FBGC formation and the FBR offers the dual promise of facilitating the development of strategies to improve the function and longevity of biomaterials as well as enhance our fundamental understanding of key cellular processes. Identification of required molecules should lead to the rational design of biomaterials with the capacity to modulate their expression and/or function in a beneficial manner. Current approaches are predominantly focused on surface modifications that have had limited success in curbing the FBR and in general apply to a small subset of biomaterials. In contrast, molecular approaches could be applied in numerous applications. Equally important, the elucidation of the molecules and pathways that regulate FBGC formation should provide insights relevant to other types of cell fusion including osteoclast and myoblast formation. Similarly, a more in-depth understanding of the processes that regulate the encapsulation of biomaterials could enhance our ability to combat other fibrotic diseases.

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Fig. 7.1.

Overview of macrophage participation in the foreign body response. Macrophages are recruited to the site of implantation where they make contact with protein-coated biomaterial surfaces leading to their unique activation. A subset of macrophages engages in cell-cell interaction leading to fusion and formation of foreign body giant cells. Both activated macrophages and foreign body giant cells provide pro-fibrotic signals that result in the encapsulation of biomaterials. Molecules that were shown to influence the progression of the FBR in genetically modified mice are shown

Fig. 7.2.

Biomaterial-induced inflammasome activation. Nano- and micro-sized particles can induce assembly of the inflammasome complex leading to conversion pro-caspase 1 to caspase 1, which then converts pro- IL-1 β to IL-1 β . Secretion of IL-1 β contributes to the inflammatory response. Small size particles induce activation following uptake by macrophages and lysosomal rupture. In addition, certain particles can induce activation via the generation of reactive oxygen species and/or K⁺ efflux. Large biomaterials, too big to be taken up by cells, can also induce inflammasome activation and IL-1 β secretion via a process that depends on membrane dynamics. However, the exact mechanism of activation has not been elucidated

Fig. 7.3.

JAK/STAT and NFκB pathways in biomaterial-adherent macrophages. Exposure of biomaterial-adherent macrophage to IL-4 induces activation of the JAK/STAT pathway leading to phosphorylation of STAT6 that translocates to the nucleus where it promotes the expression of genes including E-cadherin and β-catenin. A separate more complex and not completely defined IL-4-induced pathway causes upregulation of MCP-1 and TNF, which are secreted and bind their respective receptors. TNF activates the canonical NF-κB pathway by phosphorylation of IκB by IKK and its subsequent ubiquitination and degradation.

Degradation of I κ B leads to liberated p50/p65 complex that translocates to the nucleus and induces transcription. Both pathways are essential for macrophage fusion in vitro and the process depends on IL-4 and interaction with specific surfaces

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