**Toll-Like Receptors and the Host Response to Biomaterials**

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The host response to a biomaterial is an innate immune response in which the biomaterial is seen as a foreign entity. This involves aspects of an inflammatory response which biomaterial scientists have studied for many years such as opsonization and complement activation, resulting in leukocyte recognition of the biomaterial using cognate receptors. This leukocyte adhesion and activation results in effector functions such as release of proteolytic enzymes, respiratory burst and cytokine release. In this way, the body attempts to destroy the invading foreign entity and control the inflammatory and wound healing responses.

Macrophages and dendritic cells (DCs) are key cellular players in the innate response to a foreign entity – DCs are unique in their ability to stimulate naïve T cells in an adaptive immune response. Macrophages and DCs use pattern recognition receptors (PRRs) such as complement receptors, scavenger receptors, C-type lectin receptors and toll-like receptors (TLRs) to recognize and respond to pathogens. During an innate immune response, DC respond to pathogen-associated molecular patterns (PAMPs) through PRRs, most notably using TLRs, whose signaling through NF-κB, results in maturation of DCs. Mature DCs are then fully equipped to stimulate T cells with antigens that they picked up during the innate immune response. In this way, DCs provide a link between innate and adaptive immunity. Adaptive immune responses in the context of biomaterials are particularly noteworthy when there is an associated antigen, particularly the biological component of combination products. TLR4 is the known receptor for PAMPs such as lipopolysaccharide (LPS) and therefore links LPS to its adjuvant effect. In addition, TLR4 can also recognize endogenous molecules or 'danger signals' such as HSP60 to induce its natural adjuvant effect. In this way, tissue damage can modulate an adjuvant effect.

Since biomaterials are used as vehicles for biological components in combination products, it is important to clarify the role of the biomaterial in potentiating any immune response towards the biological component due to a biomaterial adjuvant effect. We have shown that poly(lactic-co-glycolic acid) (PLGA) acts as an adjuvant in the immune response against co-delivered antigen. As adjuvants act through the maturation of DCs, we have determined a differential biomaterial effect on DC maturation. Specifically, chitosan or PLGA films induce DC maturation, while alginate or agarose films do not and hyaluronic acid films inhibit DC maturation.

We are examining the PRRs which may be involved in mediating DC recognition of and response to biomaterials, particularly focusing on TLR4 and C-type lectin receptors. We are also characterizing the biomaterial-associated carbohydrate patterns which may be recognized by the PRRs. DCs may recognize and respond to biomaterials either indirectly through carbohydrate modifications of adsorbed proteins or through carbohydrates inherent in the biomaterial structure using PRRs to initiate an innate immune response. Self assembled monolayers (SAM) of defined chemistries were associated with differential carbohydrate profiles in the adsorbed protein layer. $NH_2$ SAM surfaces had highest amounts of carbohydrates and $CH_3$ SAM surfaces the lowest. DCs cultured on $NH_2$ and COOH SAM surfaces were most mature. DCs cultured on $CH_3$ SAM were least mature, while in the presence of highest proinflammatory cytokine levels, due to apoptosis and immunosuppressive effects. Preliminary results suggest that culture with de-glycosylated FBS slightly increased background iDC maturation, but strikingly lowered LPS maturation effects and overwhelmed any biomaterial effects on maturation marker expression. This suggests that the nature of the surface carbohydrates can have a profound effect on biological responses.

DCs derived from $TLR4^+$ (C57BL/10ScSnJ) mice showed increased expression of CD86 upon treatment with PLGA films, and this was absent for DCs derived from $TLR4^-$ mice (C57BL/10ScNJ). Hence, TLR4 has been shown to play a role in PLGA-induced DC maturation.

To pinpoint gene-regulating factors of the biomaterial/TLR4 interaction, transcription factor (NF-κB and AP-1) activation and resultant interleukin-8 release were analyzed with ELISAs using a model *in vitro* system utilizing a stably-transfected HEK293 line expressing human-TLR4/MD2/CD14 treated with PLGA or agarose films or with LPS as a positive control. While LPS signaled through NF-κB, biomaterials did not. Interestingly, LPS, PLGA and agarose activated most AP-1 family members. This result is an indication of how a biomaterial may be a different agonist of leukocyte activation than a pathogen.

In summary, biomaterials can differentially affect DC maturation. DCs may use mechanisms analogous to pathogen recognition to respond to biomaterials (e.g. TLRs). Protein glycosylations are important for immune cell interaction with a biomaterial. Biomaterials are different from LPS in their effects on DCs (through TLR4 signaling). Avoiding biomaterial implant site associated 'danger signals' may minimize immune responses to tissue engineered constructs. These studies provide insight into design criteria as well as immunomodulatory strategies for biomaterials for a range of applications.

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