

## The Role of Toll-like Receptor 4 in Biomaterial-induced Dendritic Cell Maturation

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**Introduction:** Biomaterials in the context of biologics (protein, DNA, cell)-delivering scaffolds for tissue regeneration or carriers for vaccine delivery rely heavily on the ability to minimize or maximize, respectively, the subsequent adaptive immune response to the delivered biologic. The driving force for this response stems from the actions of dendritic cells (DC) which function as potent antigen presenting cells. During the innate immune response, adjuvants induce maturation of DC such that they efficiently stimulate T-cells by causing upregulation of costimulatory and major histocompatibility [MHC] class I and II molecules.

We have previously shown that biomaterials [such as poly(lactic-co-glycolic acid) (PLGA) films or microparticles (MPs)] act as adjuvants<sup>1,2</sup> by inducing maturation of DC<sup>3</sup>. Bone marrow derived DC from C57BL/6 mice have also shown similar maturation in the presence of PLGA films or MPs<sup>4</sup> by increasing maturation marker expression and pro-inflammatory cytokine secretion.

The goal of this work is to identify how DC recognize and respond to biomaterials. During an innate immune response, DC respond to pathogen-associated molecular patterns (PAMPs) through pathogen recognition receptors [among them Toll-like receptors (TLR) and C-type lectin receptors] which induce DC maturation. Toll-like receptor 4 (TLR4) is the known receptor for PAMPs such as lipopolysaccharide (LPS) and therefore links LPS to its adjuvant effect. Endogenous molecules have also been linked to TLR4 activation (e.g., HSP60<sup>5</sup>) to induce their adjuvant effect leading to our hypothesis that biomaterials may also require TLR4-mediated signaling. Here we present evidence that biomaterial-induced DC maturation requires TLR4. Hence, biomaterials may subsequently be designed to avoid or enhance TLR4 activation based on required needs of the delivered protein/vaccine.

**Methods:** PLGA (75:25) films were prepared by solvent casting and immediately prior to experiment, were exposed to UV for 30 min on each side. Endotoxin content of films was assayed (QCL-1000 LAL assay, Cambrex) and measured below 0.1 EU/mL (the lower limit). PLGA MP (3.8  $\mu$ m) were prepared by a double emulsion solvent extraction technique with final rinses in endotoxin-free water and UV exposure (1 hr) immediately prior to DC treatment at a 5:1 MP:cell ratio. Endotoxin content of MP was assayed and was not detectable (below blank).

Bone marrow derived DC were derived from mice lacking TLR4 expression, C57BL/10ScNJ (TLR4<sup>-</sup>), and mice with wild-type TLR4, C57BL/10ScSnJ (TLR4<sup>+</sup>) (both from The Jackson Laboratory). TLR4<sup>-</sup> and TLR4<sup>+</sup> mice were age-matched and 6-8 weeks at time of sacrifice (6 mice per group). Bone marrow was harvested, processed and cells resuspended (1.5\*10<sup>6</sup>/mL) in media containing 20ng/mL of both GM-CSF and IL-4. On day 6 of culture, loosely adherent cells were collected and CD11c<sup>+</sup> cells isolated using magnetic beads for immature DC (iDC). These cells were

plated (10<sup>6</sup> cells/mL) and treated with 1  $\mu$ g/mL ultra-pure LPS (Invivogen), PLGA films or PLGA MP (24 hrs) or left untreated as iDCs. Loosely adherent cells were collected and flow cytometry was performed. DC markers I-A<sup>b</sup>, CD11c, CD80, CD86 as well as phosphatidylserine and propidium iodide were used to examine both the maturation and apoptotic effects of biomaterials on DC. To pinpoint gene-regulating factors of the biomaterial/TLR4 interaction, transcription factor activation (both NF- $\kappa$ B and AP-1) was analyzed using a model *in vitro* system utilizing a stably-transfected HEK293 line expressing human-TLR4/MD2/CD14 (Invivogen). Cells (20\*10<sup>6</sup>) were treated with 1  $\mu$ g/mL LPS, PLGA 75:25 films or agarose films. At 5 and 24 hr time points, cells were harvested by scraping and nuclear extracts isolated and assayed using ELISA-based techniques for NF- $\kappa$ B and AP-1 family member activation. IL-8 ELISAs were performed on supernatants.

**Results/Discussion:** DCs derived from TLR4<sup>+</sup> mice display increased expression of CD80 and CD86 when treated with LPS while DCs derived from TLR4<sup>-</sup> mice show no difference as compared to iDCs. DCs derived from TLR4<sup>+</sup> mice showed increased expression of CD86 upon treatment with PLGA films, and this was absent for DCs derived from TLR4<sup>-</sup> mice. For DCs derived from TLR4<sup>+</sup> (but not from TLR4<sup>-</sup> mice) mice, phosphatidylserine (PS) on DC surface was decreased as compared to iDC when treated with LPS but increased in response to film and MP. Thus, treatment with PLGA films or MPs causes DC apoptosis (only MP show slight decrease in viability). For HEK TLR4/MD2/CD14 cells, all NF- $\kappa$ B family members were found to be activated by LPS treatment but not by PLGA or agarose films. Accordingly, only LPS caused an increase in IL-8 secretion. Preliminary results indicate that LPS, PLGA, and agarose treatments activate most AP-1 family members at 24 hr time point.

**Conclusions:** TLR4 has been shown to play a role in PLGA-induced DC maturation. It has also been shown to play a role in DC apoptosis stimulated by PLGA films and MP. Future work will include elucidating if biomaterials are triggering a Th2 response and if this response is mediated through the differential transcription factor activation.

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**References:** 1. Matzelle, M. Babensee JE, Biomaterials, 2004, 25: 295-304. 2. Bennowitz NB, Babensee, JE, Biomaterials, 2004, 26: 2991-2999. 3. Yoshida, M, Babensee, JE, J Biomed Mater Res, 2004; 71A: 45-54. 4. Yoshida, M, Mata, J, Babensee JE, J Biomed Mater Res, in press. 5. Vabulas, RM et al, J Biol Chem 2001; 276(33):31332-9.