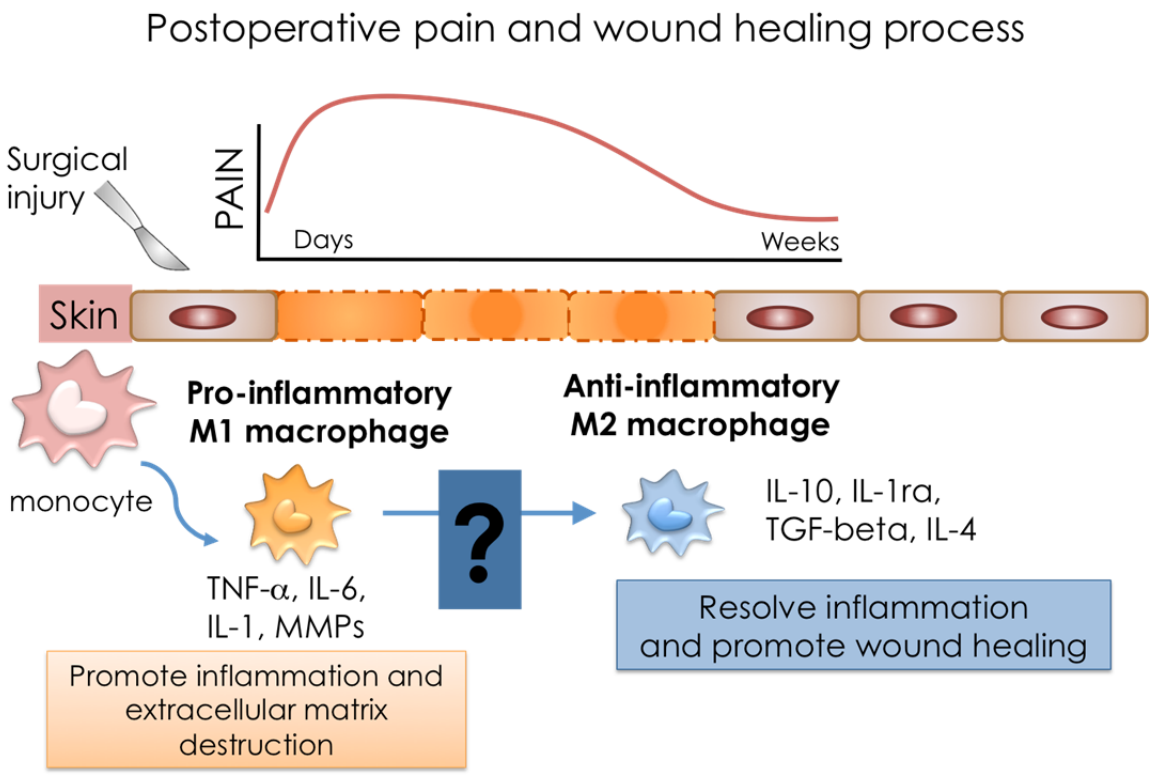
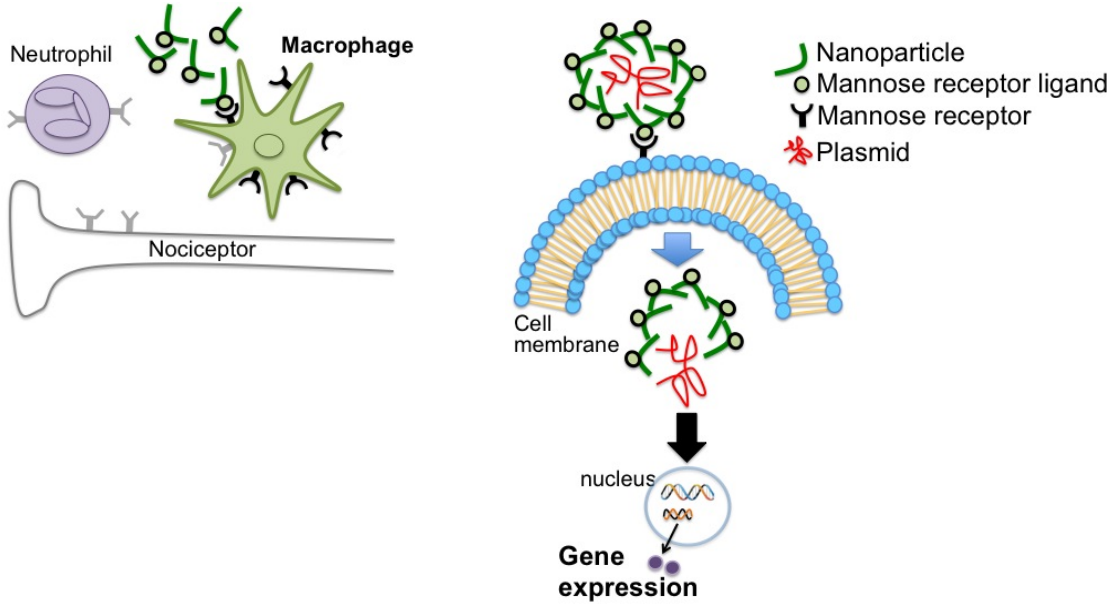
With grant grant GM109333 from the National Institute of General Medicine Sciences, we are studying the role of CD163 in the normal resolution of pain after surgery and its potential therapeutic effects to prevent or treat chronic postsurgical pain. Under this initial hypothesis, our studies have originated multiple projects encompassing a multidisciplinary approach.

1) Our efforts are focused on developing a therapeutic strategy following a peripheral route of administration to facilitate its potential application in the clinic. Macrophages are peripheral immune cells involved in local inflammation following surgical tissue damage (M1 phenotype). Macrophages also, through not completely understood mechanisms, adopt an alternative phenotype (M2) to promote the resolution of inflammation and tissue repair. This process parallels the development, maintenance, and resolution of pain. Therefore, our goal is to specifically target macrophages to induce an efficient and timely tissue repair and inflammation resolution.

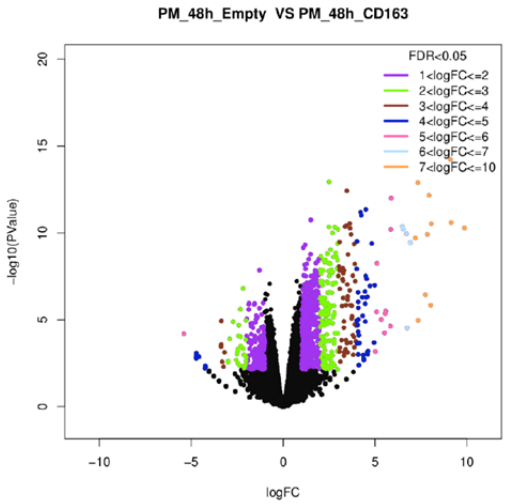


2) We hypothesize that CD163 promotes the transition from M1 to M2 phenotypes in macrophages. To test this hypothesis we are inducing the expression of CD163 gene in macrophages using nanotechnology. We are currently utilizing a nanoparticle grafted with a mannose receptor ligand. Since monocytes are the only cells that express mannose receptors, this nanoparticle preferentially targets macrophages. This nanoparticle is utilized as our plasmid carrier to

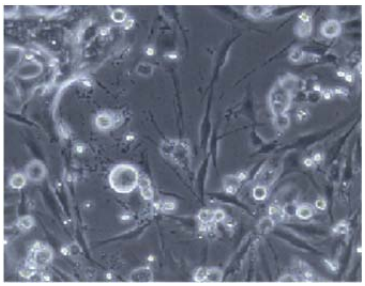
induce CD163 in macrophages, avoiding the induction of this gene to other cells (i.e. neutrophils or neurons).

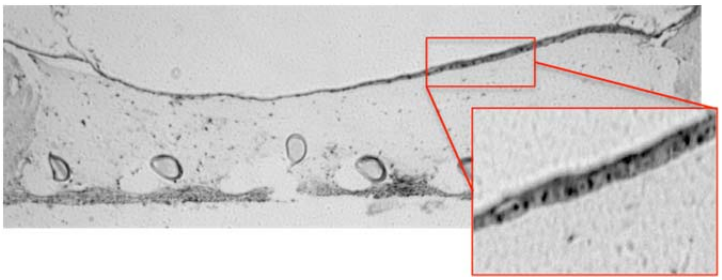


3) Our studies include functional assays to determine whether the induction of CD163 using this approach promotes an M2 cellular phenotype in human macrophages. These assays include the measurement of pro- and anti-inflammatory factors and determination of changes in intracellular signaling pathways. Our studies include metagenome analyses in human macrophages under specific conditions to identify novel potential molecular targets that are related to tissue repair and/or inflammation. The scientific premise of our hypothesis is that by promoting an M2 macrophage phenotype we will prevent or revert peripheral nociceptor sensitization, and, subsequently, central changes that underlie the development of chronic pain.

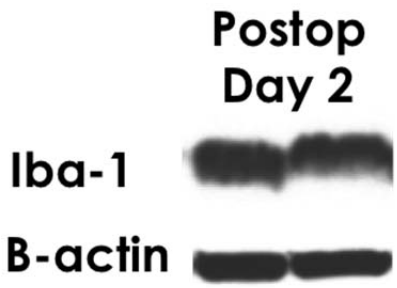


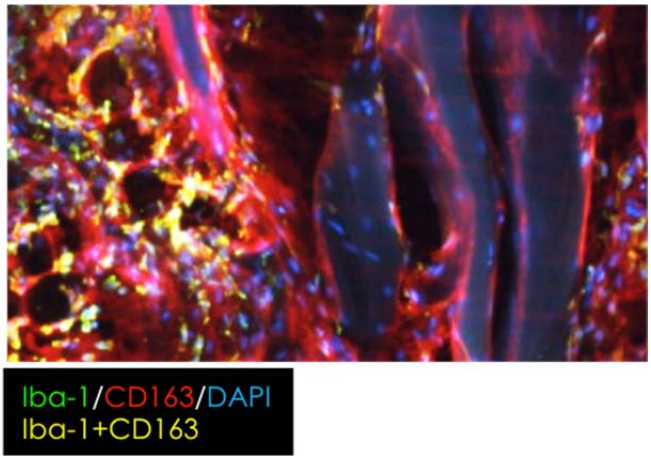
4) We are interested in understanding the cellular and biochemical interactions between macrophages and skin cells and how these processes orchestrate wound healing. Specifically, we utilize *in vitro* keratinocyte and fibroblast wound healing assays, and an ex vivo 3D human organotypic wounded skin tissue assay to explore the role of macrophage CD163 in skin cell function.





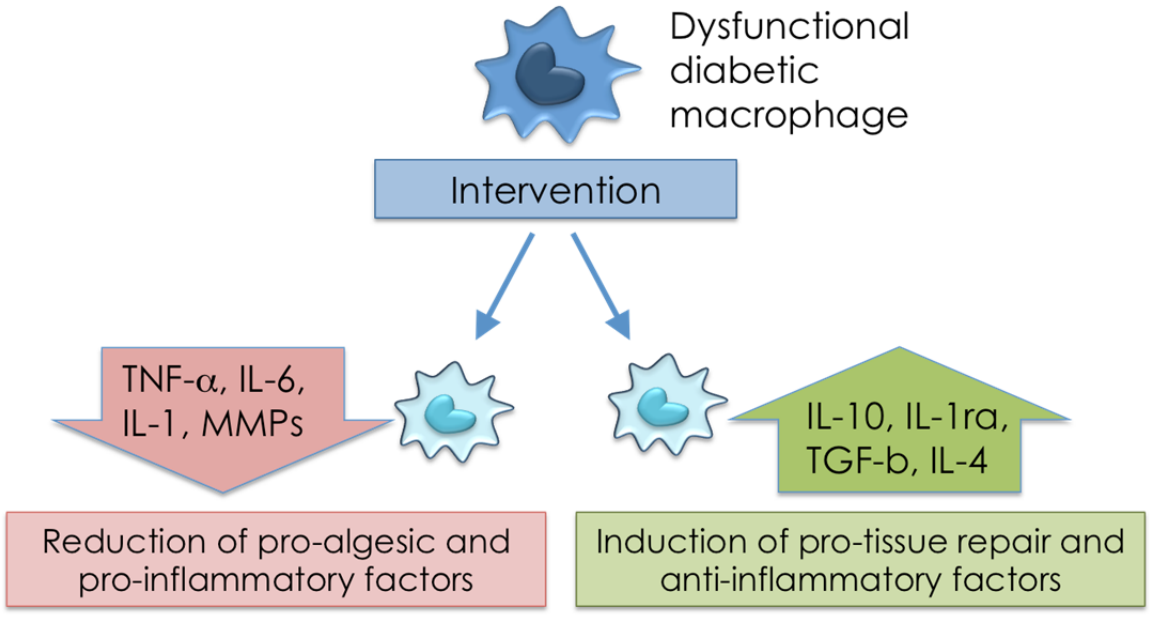
5) To further test our hypotheses *in vivo*, we use rat models of postoperative pain. The skin/muscle incision and retraction (SMIR) results in evoked mechanical hypersensitivity and spontaneous weight bearing changes (indicative of ongoing non-evoked pain) that last for two or three weeks. This rat model of persistent postsurgical pain is ideal to test our hypothesis that the induction of CD163 in local macrophages promotes a more efficient and faster resolution of inflammation and postoperative pain. Behavioral studies for evoked and spontaneous pain, molecular assessments at the mRNA and protein level, histopathologic analyses, and comprehensive statistical tests comprise our methodologies and approach.





6) Due to the major role of macrophages in inflammation and wound healing, we are also studying their function and capabilities under diabetic conditions. Diabetic ulcers are one of the major complications of peripheral diabetic neuropathy, and the major cause of amputations. It is well known that

macrophages from patients with diabetes are dysfunctional and display a persistent pro-inflammatory like phenotype. We are exploring multiple molecular and genetic approaches to restore macrophage normal functions under a hyperglycemic environment. Our ultimate goal is to develop a therapeutic approach to promote a better tissue repair for diabetic ulcers, and potentially reduce the macrophage pro-inflammatory state found in painful diabetic neuropathy to treat this neuropathic pain condition.



Macrophages with restored functions and capabilities