



2009 Research Grant Program Winning Abstract

Immunity to Bacterial Biofilm

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Specific Aim

The long-term goal of our research is to better characterize the cellular and molecular mechanisms of the wound healing process. Despite extensive research addressing the normal wound healing process, little is known about how the bacterial biofilms can disrupt this process, leading to the development of a chronic wound. We hypothesize that bacterial biofilms mediate specific pathological effects against host innate immune cells in the wound environment resulting in a deviation from the normal wound healing process. We propose to address this hypothesis by characterizing monocyte/macrophages response to bacterial biofilm and determining how phenotypic manipulation of this cell population affects the innate immune response to bacterial biofilm. The studies proposed here may uncover not only the mechanisms at the root of the deviation from the normal healing process that results in the development of a chronic wound, but also may uncover novel means of therapeutics through the manipulation of phenotype. Therefore, these studies have a direct translational aspect that may add to the clinical toolbox when facing the increasing threat of chronic wounds.

Background

The normal wound healing process proceeds through a predictable series of stages. At each stage, specific cell types mediate specific roles in the normal healing process. In the normal wound healing process, the wound will transition from early to late stage inflammation characterized by a shift in population from neutrophils to macrophages. Although macrophages, like neutrophils, do participate in debridement and the inflammatory process, they are also major contributors to the wound healing process via secretion of cytokines, chemokines, and growth factors. The initial role of the macrophage in the wound is the removal of apoptosing neutrophils stimulating the resolution of inflammation and progression to proliferation; however, in a chronic wound macrophages fail to dispose of apoptotic neutrophils leading to host tissue damage. Indeed, under sterile conditions it was demonstrated that neutrophils did not perturb the wound healing process; however, macrophage depletion resulted in failed debridement and a disrupted healing process. Macrophages also appear to play an important role in mediating the wound healing process beyond resolution of inflammation. It is our hypothesis that this later role of macrophages is the result of a phenotypic shift of the wound macrophages and that this shift is essential to the normal wound healing process.

Macrophages are generally characterized as either M1 or M2 macrophages. The classically activated (M1) macrophages are stimulated by interferon-gamma or lipopolysaccharide. These macrophages secrete tumor necrosis factor, are highly microbicidal, and are pro-inflammatory. M1 macrophages are primarily associated with protection during acute infection and persistence of these cells can prove detrimental. M1 polarization results in chronic inflammation with associated necrosis, fibrosis, and scar formation. Alternatively activated macrophages (M2) have been associated with tissue remodeling and secrete growth factors that stimulate fibroblast proliferation,



migration, and extracellular matrix formation. Furthermore, M2 macrophages secrete transforming growth factor-beta that can stimulate keratinocyte migration, heparin-binding epidermal growth factor secretion, and transforming growth factor-alpha secretion leading to keratinocyte migration and proliferation. Finally, M2 macrophages may contribute to neovascularization via secretion of VEGF and both basic and acidic FGF stimulating endothelial cell proliferation.

Methods

We intend to address our hypothesis using a co-culture model based on a co-culture model developed in our lab. Briefly, we will co-culture primary monocyte/macrophage populations with single species bacterial biofilms including the major wound pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Characterization of innate immune response to the bacterial biofilm will be assayed via cytokine, chemokine, and growth factor detection and inflammatory marker detection. To control for activation and as a basis of comparison, innate immune cells will be artificially stimulated and assayed for chemokine and inflammatory markers. Using immunohistochemistry, we will characterize the phenotype of the primary monocyte/macrophages in the presence of the bacterial biofilm. Finally, we intend to differentiate our primary monocytes into M1 or M2 subtype macrophages and characterize the difference in response by these two phenotypically distinct populations to bacterial biofilm.

Significance

Chronic wounds, including diabetic foot ulcers, pressure ulcers, and venous leg ulcers, have received a sadly minimal amount of attention both in the press and in the research literature. However, as the United States population ages and more Americans develop obesity related diabetes, the related increase in chronic wounds can no longer remain under the radar. In 2007 nearly 18 million Americans had developed diabetes with an associated cost to the American public of \$174 billion in health costs. Nearly 15% of diabetics can be expected to develop lower extremity ulcers and in 2007 two percent of the American population experienced a non-healing wound.

Here we propose a comprehensive characterization of the monocyte/macrophage response to bacterial biofilm. Furthermore, we propose to utilize our understanding of the normal wound healing process to manipulate macrophage phenotype with the end-goal of obtaining translational research to enhance the treatment of chronic wounds. Taken together, these studies will further our understanding of the wound healing process, how deviations from the wound healing process occur, and suggest potential therapeutic target upon which further translational research should focus.

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