Colonizing Bacteria Impacts Local Host Response to Inflammation and Wound Healing for Burn Wounds: A Preliminary Burn Wound Microbiome Analysis

Bonnie C. Carney BS, Pranay R. Randad MS, Rachel T. Ortiz MS, Lauren T. Moffatt PhD, Jeffrey W. Shupp MD

The Burn Center, Department of Surgery, MedStar Washington Hospital Center, MedStar Health Research Institute, Washington DC

Introduction

Challenges to burn wound healing include wound depth progression and the development of hypertrophic scar. While many wounds do not become invasively infected, all wounds are colonized. Although different strains and species of bacteria are unique, there exists a set of molecules that are highly conserved among classes of microbes. These are known as pathogen-associated molecular patterns (PAMPS) and their receptors on host cells are called pattern-recognition receptors (PRRs). PRRs allow the host to recognize microbes and lead to the subsequent signaling cascade known as the innate immune response and secretion of pro-inflammatory cytokines. Each sub-group of PRRs possess a certain specificity for ligands. The presence of and demographic make-up of microflora may perturb the local host immune response, as well as play a critical role in the inflammatory processes of wound healing. It is essential to understand the role that colonizing bacteria may play in the wound healing process. To date, there have been no published reports that characterize the microbiome of a healing burn wound and elucidate its role in normal versus abnormal wound healing.

Methods

Day 0
1. Skin sterilized with ChloraPrep®
2. Baseline biopsies collected from uninjured skin
3. Paired 2 cm x 2cm burn wounds created with aluminum billets on the dorsum of Sprague Dawley rats (Figure 1)

Day 1
Wounds **were not** inoculated with bacteria or treated with dressing

Days 2,4,6,8,10: Biopsies taken from the **clean, un-inoculated** wound site

- DNA isolated from biopsies → 16s rRNA primers designed to amplify a homologous region of the 16s rRNA bacterial gene used in qPCR to determine bacterial bioburden (Figure 2)
- RNA isolated from biopsies → RNA samples and gene specific primers used in real time RT-PCR to determine transcript levels of host Toll-like receptor 1 (TLR1), TLR2, and NLRP3 (Figure 3)
- Quantitative culture analysis performed to determine bacterial levels

Bacterial bioburden was quantified using a standard curve constructed from an analogous amplification of serial dilutions of known quantities of DNA isolated from MRSA (Figure 2 inset; Nadkarni et al). Digital photos were taken of all wounds at all time points. Images were randomized and an expert reviewer used a novel photo grading scale to quantify wound healing (Table 1). Results for each animal were averaged and plotted over time (Figure 4).

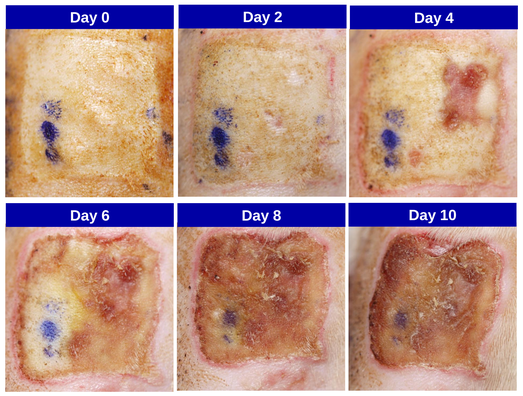

Figure 1. Representative digital pictures of the right wound over the time course

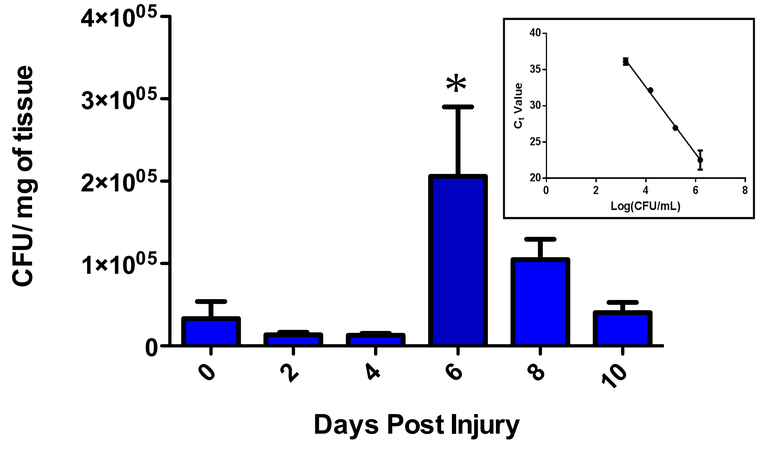

Figure 2. Bioburden measurement shows a peak at Day 6 post-injury (* p<0.02). Inset: Bioburden was quantified using a standard curve as described in the methods

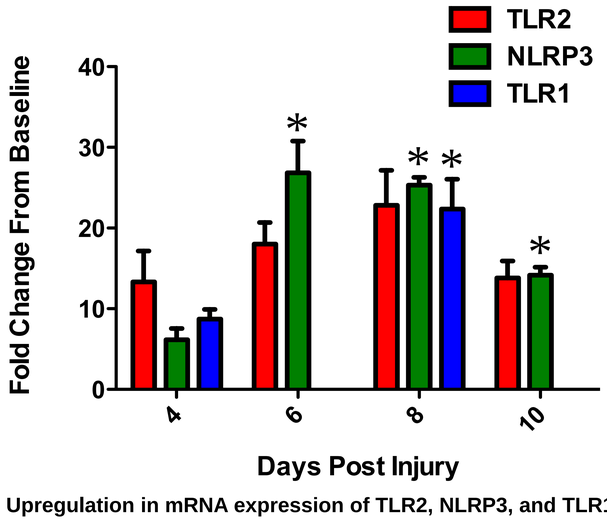

Figure 3. Upregulation in mRNA expression of TLR2, NLRP3, and TLR1 (* p<0.05)

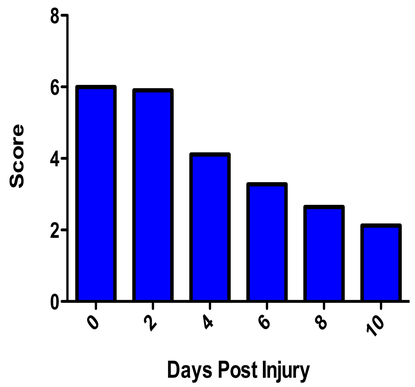

Figure 4. Scoring of pictures depicts wound healing over the time course

Results and Discussion

Quantitative culture showed **no bacterial growth** from skin biopsies over the time course. Since this traditional method of bacterial quantification was not sufficient, a more sensitive method was used. Quantification of 16s rRNA indicated a slight decrease in burn wound bioburden on Day 2 post injury, followed by a steady increase in bioburden throughout the 10 day time course (Figure 2). Burn wound bioburden peaked at Day 6 post injury, when it was significantly greater than the level at Day 2 (n=5, p<0.02). Burn wound transcript levels of TLR2, NLRP3, and TLR1 were up-regulated on Days 4-10 over the baseline levels (Figure 3). TLR2 peaked at 22.83 fold increase from baseline at Day 8 post injury (n=3). Levels of NLRP3 on Days 6, 8, and 10 were significantly greater from levels at Day 4 (n=3, p<0.01) and peaked at a fold change of 26.87 at Day 6 post injury. Day 8 levels of TLR1 were significantly upregulated from the levels at Day 4 and peaked at a fold change of 22.34 at Day 8 post injury (n=3, p<0.05). These results suggest that a correlation exists between local host innate immune response to burn injury and bacterial colonization of the burn wound. The innate immune response may play a critical role in the wound healing process.

Score	% Dry Contracted Scab	% Edematous Eschar
1	100	0
2	99-75	24-1
3	74-50	49-25
4	49-25	74-50
5	24-1	99-75
6	0	100

Table 1. Scoring system for the digital picture grading scale

Future Direction

A more in depth analysis is needed in order to characterize the composition of the burn wound microbiome over the time course. The same DNA isolated from burn wound biopsies will be used to sequence the 1400 base pair 16s rRNA gene amplicon. A microbiomic profile will allow for the characterization of microflora that contribute to deranged wound healing and the initiation of the innate immune response leading to prolonged inflammation. This characterization of the burn wound may have predictive power in categorizing wounds that will heal normally versus those that will heal abnormally. Furthermore, in understanding this concept, antibiotic treatment may be tailored in a wound-specific manner.

Reference Cited: Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology. 2002;**148**(1):257-66.