INTRODUCTION

Post-traumatic heterotopic ossification (HO) occurs in over 60% of patients with time-of-war traumatic amputation [1], although the etiology of HO is unclear. Traumatized muscle contains mesenchymal progenitor cells (MPCs) [2], and dysregulation of these cells by traumatic and wound healing factors may lead to osteogenic differentiation with subsequent ectopic bone formation [3]. The cytokine expression profile in injured muscle has not been well characterized, so it is unknown what specific factors may be enhancing the osteogenic potential of the MPCs. Therefore, the goal of this study was to determine which cytokines are differentially expressed in traumatized muscle. Our hypothesis was that a subset of cytokines that enhances osteogenic behavior of progenitor cells was up-regulated in traumatically injured muscle tissue. Our specific aims were (1) to identify the cytokines up-regulated in the injured muscle, and (2) to determine the cellular localization of these cytokines within the traumatized muscle.

MATERIALS AND METHODS

Tissue samples of debrided muscle were obtained during serial washouts of traumatic time-of-war extremity wounds (n=7). Control muscle tissues were obtained during routine anterior cruciate ligament reconstruction when autograft tendons were harvested (n=5). All tissue samples were obtained at the Walter Reed Army Medical Center with IRB approval. Tissue samples were dissected to remove any fibrous, fatty or necrotic regions and then flash frozen in liquid nitrogen. The frozen tissue was homogenized for TRizol-based RNA extraction. Gene expression was determined using real-time RT-PCR arrays (SABiosciences) to simultaneously assay for over 80 common cytokine genes and receptors, which included interleukins, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), and transforming growth factor β (TGF-β) family members. Cytokines and cellular localization was carried out using immunohistochemistry on tissue samples that were fixed, paraffin embedded and sectioned at 5µm thickness. CD105 was used as a cell surface marker to identify MPCs. Histology was done using Hematoxylin-Eosin (H&E) and Mallory’s Trichrome stain.

RESULTS

A differential cytokine expression profile was seen between traumatized and non-traumatized muscle. The traumatized muscle significantly up-regulated a sub-set of cytokines associated with osteogenic induction (e.g., BMP-1 and TGF-β), compared to the untraumatized muscle (Fig. 1). BMP-4, a cytokine associated with genetic forms of HO (i.e., Fibrodyplasia Ossificans Progressiva [3]) was down-regulated. The traumatized muscle contained damaged muscle fibers, infiltrating mononuclear and fibroblastic cells (including CD105 positive cells) and fibroproliferative lesions (indicated by the deep blue Trichrome staining; Fig. 2). Immunohistochemical analysis indicated that the osteogenic cytokines were expressed in the same regions of the tissue as the CD105 positive cells. Neither CD105 nor the osteogenic cytokines were detected in the untraumatized tissue.

DISCUSSION AND CONCLUSIONS

Our results have identified a set of genes that are up-regulated in traumatized muscle and may play a role in producing ectopic bone after traumatic injury. Although BMP-2 and BMP-4 were not up-regulated by trauma, other cytokines might also enhance osteogenesis in the injured tissue. For example, BMP-1 is significantly up-regulated in the traumatized muscle, and it interferes with the function of chordin, an antagonist of BMPs [4], which may increase the efficacy of BMP-2 or -4 within the tissue to stimulate osteogenesis. In addition, CD105 (endoglin), which was used in this study as a marker for the MPCs, is a cell surface protein that regulates the activity of TGF-β receptors [5]. CD105 positive cells were present near and inside the fibroproliferative regions, which co-localized with TFG-β expression. These data suggest that the traumatized muscle contains subsets of tissue that are conducive to intramembranous and endochondral bone formation. Post-traumatic HO likely has a multi-factorial etiology, and this study characterizes cellular and genetic differences between traumatized and non-traumatized muscle tissue that may play a role in the disease process. We are using the findings from this study as a foundation to further study the mechanisms that lead to HO following musculoskeletal injury.

REFERENCES:

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