Wounds and Innate Inflammatory Responses

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Brita Pukstad, at St. Olav's Hospital in Trondheim, Norway, defended her doctoral thesis "Characterization of innate inflammatory responses in acute and chronic wounds" at the Norwegian University of Science and Technology (NTNU) on December 16th, 2011. Her supervisors were Terje Espevik, NTNU and David W. Thomas, University of Wales. The evaluation committee members and opponents were Ralf R. Schumann, Charité-Universitätsmedizin Berlin, Robin Ingalls, Boston University School of Medicine, and Arne Sandvik, NTNU.

The main aim of the study was to achieve a better understanding of the inflammatory responses involved in wound healing, and the participation of Toll-like receptors (TLRs) in these processes.

In innate immunity, TLRs have an important role in recognizing foreign molecular patterns derived from microbes, and in recognizing altered cellular components when tissue has been damaged. They sense danger, and contribute to the initiation of inflammation upon skin injury. It is already known that TLR3 is stimulated by the release of nucleic acids (dsRNA) from damaged cells, and so is involved in acute injury of the skin. In chronic wounds, however, little is known about the involvement of TLRs. Chronic wounds are not uncommon among the elderly, and is becoming a problem with high economic impact in industrialized countries.

We analyzed wound fluid from chronic leg ulcers due to venous insufficiency, and characterized cytokines, TLR responses, and the antibacterial peptide Lipocalin-2. By comparing wound fluid from healing and non-healing wounds we found high TLR2- and TLR4-activity to be a feature of non-healing, together with high levels of Lipocalin-2 and pro-inflammatory cytokines. The wounds included in the study were not clinically infected, and so the sustained TLR- and Lipocalin-2-activity supported the theory of increased inflammation as being a part of the non-healing property of chronic wounds. Further, we found Lipocalin-2 to be a potential biomarker of the healing status of chronic wounds.

We also analyzed cytokine profiles in wound fluid from acute wounds. In parallel with this, we investigated supernatants from cells normally present in a wound bed. The cells were stimulated with different TLR ligands, and with inflammatory molecular mediators such as TNF-α, IL-1β, IL-6 and IL-10. The cells involved were keratinocytes, endothelial cells, fibroblasts, monocytes, and neutrophil granulocytes. We characterized 27 molecular mediators (cytokines and growth hormones) in acute wound fluid 24 h after skin injury and in cell supernatants, and clarified which cells produce which mediators. Finally, we con-

Fig. 1. From left to right: Magne Børset (acting dean), Brita Pukstad, Robin Ingalls, Arne Sandvik, and Ralf Schumann.

centrated on keratinocytes and their role in skin injury through detection of dsRNA. This cell type was earlier thought to be just a mechanical supportive cell, giving the skin its structure, and a physical protective barrier. In recent years it has been clear that keratinocytes have important roles in skin inflammation and in protection against microbes. They are also able to detect injury of other cells through stimulation of TLR3.

In our study, we found stimulation of TLR3 by the synthetic dsRNA analogue, polyI:C, to initiate a powerful inflammatory response in keratinocytes. We also found polyI:C to be toxic to these cells. Co-stimulating the keratinocytes with polyI:C and another TLR ligand, the TLR9 ligand and oligodeoxynucleotide CpG, protected the keratinocytes from this toxic effect and reduced the inflammatory response. We found that the protective effect was dependable of the timing of introduction of CpG, and that this was due to a competitive uptake of CpG and polyI:C into the cells.

The immunomodulatory response of CpG is known, and its potential in treating diseases has already been pursued in cancer, allergy, infection and dermatitis. A possible wound-healing effect of CpG has also been proposed. The cytoprotective effect of CpG on keratinocytes, though, is a new and potential finding. In this regard, prevention of UV-induced cell damage is possible and should be pursued further.

More knowledge about the intricate systems of immune responses and regulation in wound healing, and how they lead to the repair of injured skin is of great importance. Most of all, this knowledge will lead to a better understanding of the pathogenesis of non-healing, chronic wounds.

List of publications

1. Pukstad BS, Ryan L, Flo TH, Stenvik J, Moseley R, Harding K, et al. Non healing is associated with persistent stimulation of the innate immune response in chronic venous leg ulcers. J Dermatol Sci 2010; 59: 115–222.
2. Grimstad Ø, Sandanger Ø, Ryan L, Otterdal K, Damås JK, Pukstad B, Espevik T. Cellular sources and inducers of cytokines present in acute wound fluid. Wound Rep Regen 2011; 19: 337–347.
3. Grimstad Ø, Pukstad B, Stenvik J, Espevik T. Oligodeoxynucleotides inhibit Toll-like receptor 3 mediated cytotoxicity and CXCL8 release in keratinocytes. Exp Dermatol 2012; 21: 7–12.