

## CHAPTER 10

### IMMUNOMODULATORY PROPERTIES OF HONEY THAT MAY BE RELEVANT TO WOUND REPAIR

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Wound healing is a complex process that involves both a degenerative and reparative phase (Clarke, 1996). The tissue macrophages are cells that are crucial in regulating wound healing. Macrophages are cells derived from peripheral blood monocytes; their role involves:

- the removal of damaged connective tissue and cell debris resulting from infection or injury
- the killing and removal pathogens
- the formation of new blood vessels
- the stimulation of fibroblast proliferation that results in collagen biosynthesis that gives rise to the remodelling of connective tissue.

Macrophages are capable of synthesising and secreting cytokines, important molecules that modulate the immune response. These include Tumour Necrosis Factor- $\alpha$  (TNF- $\alpha$ ). Macrophages produce reactive intermediates (ROIs) and hydrolytic enzymes that are involved in microbial killing. They also produce growth factors and vasoactive agents which promote the repair process, attract cells to the site of tissue damage eventually leading to the removal of dead or damaged cells and tissue (Jones, 2001).

The antimicrobial and anti-inflammatory properties of honey are well established (Molan, 1999). The ability of Manuka, jellybush and pasture honey to stimulate monocytes, the precursors of macrophages, to secrete TNF- $\alpha$  has been investigated (Tonks *et al*, 2001; Tonks *et al*, 2003). The honeys were able to increase significantly the secretion of TNF- $\alpha$

and other cytokines by monocytes. The honey samples, tested as being endotoxin free, were added to cell cultures as 1% (w/v) solutions and incubated for twenty-four hours. Activation of these cells may, in part, explain the well-documented wound healing qualities of honeys such as these. Honey has the potential to aid wound healing by the ability of its glycosylated proteins or other components to stimulate cytokines such as TNF- $\alpha$  by macrophages. Glycosylated proteins are known to induce TNF- $\alpha$  secretion by macrophages, and this cytokine is known to induce wound repair mechanisms. Furthermore, the ability of honey to reduce ROI release (Tonks *et al*, 2001) may well limit tissue damage by activated macrophages during wound healing. Leakage of these components from activated cells is responsible for much of the damage to tissue that is seen after infection and tissue damage. Kasri (2004) has shown that blocking Toll-like Receptor 4 (Toll receptor: one of a family of receptors that provide a critical link between immune stimulants produced by micro-organisms and the initiation of the host defence; TLR 4 binds lipopolysaccharide LPS. Activation of the toll receptors causes the release of antimicrobial peptides, inflammatory cytokines, and molecules that initiate adaptive immunity) diminished, but did not prevent, TNF- $\alpha$  synthesis in monocytes.

An inference from this observation is that TNF- $\alpha$  release from monocytes may be elicited by more than one factor.

Inflammation is part of the normal response to infection and wounding and inflammatory signals, such as lipopolysaccharide (or endotoxin), can induce release of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) from monocytes and macrophages via cyclo-oxygenase<sub>2</sub> metabolism. PGE<sub>2</sub> modulates several inflammatory responses, increases vascular permeability and sensitivity to pain, as well as being pyrogenic. There are reports that some patients find honey soothing, but that others experience a stinging sensation. Although Al-Waili and Boni (2003) have demonstrated lowered plasma prostaglandin levels in normal individuals following oral administration of honey, a recent study (similar to Tonks *et al*, 2001, and 2003), showed that Manuka honey was able to increase significantly prostaglandin E<sub>2</sub> synthesis in monocytic cells (Morris *et al*, 2004). PGE<sub>2</sub> has been shown to be an important component in regulating wound repair in keratinocytes (Rys-Sikora *et al*, 2000).

Of the numerous cytokines produced at wound sites, the transforming growth factor-beta (TGF- $\beta$ ) superfamily has the most profound ability to influence many aspects of tissue repair. The three main isoforms (TGF  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ ) have all been localised in healing wounds. The manipulation of the ratios, particularly of  $\beta_1$  relative to  $\beta_3$ , reduces scarring and fibrosis

(O’Kane and Ferguson, 1997). TGF- $\beta$  is released by degranulating platelets, and secreted by lymphocytes, macrophages, endothelial cells, smooth muscle cells, epithelial cells and fibroblasts (Roberts and Sporn, 1996). Human cells produce all three isoforms, of which TGF- $\alpha_1$  is best-studied (Philipp *et al*, 2004). The importance of TGF- $\alpha_1$  in wound healing has been reviewed (Kim *et al*, 2005). There is currently limited evidence as to the ability of honey to regulate TGF- $\alpha_1$  synthesis. In a recent study, propolis, the resinous product collected by honey bees from plants that has been used in folk medicine since ancient times, has been shown to be able to increase TGF- $\alpha_1$  secretion by immune cells (Ansorge *et al*, 2003).

Advances in understanding the mechanisms of wound healing are of great importance in developing appropriate strategies for the clinical management of wounds. In this respect, the nuclear receptor peroxisome proliferator-activated receptor (PPAR)- $\beta/\delta$  occupies a unique position at the intersection of the pro-inflammatory and anti-inflammatory signals important in wound repair (Tan *et al*, 2005). The precise function of PPAR- $\beta/\delta$  is as yet not fully elucidated, although it is known to have a critical role in the response of keratinocytes to the inflammatory signals produced after skin injury. Although fatty acids can bind and activate PPAR- $\beta/\delta$ , studies on this isotype have so far been impeded by the lack of information about the nature of its physiological ligands and by its remarkably broad tissue distribution. The inflammation that immediately follows injury increases the expression of PPAR- $\beta/\delta$  in the wound edge keratinocytes, and triggers the production of endogenous PPAR- $\beta/\delta$  ligands that activate the newly produced receptor. This elevated PPAR- $\beta/\delta$  activity results in increased resistance of the keratinocytes to the apoptotic signals released during wounding, allowing faster re-epithelialisation.

Changes in PPAR- $\beta/\delta$  expression can have a profound effect on wound healing, and, as shown in a recent study (Tan *et al*, 2005), dictate wound repair kinetics. Following topical application of TGF- $\beta_1$  to skin wounds, a crucial dual role of TGF- $\beta_1$  as a chemoattractant of inflammatory cells and repressor of inflammation-induced PPAR- $\beta/\delta$  expression, was revealed.

The immediate response to trauma in the skin is the release of inflammatory signals. In a study (Tan *et al*, 2001) which used cultured or primary keratinocytes from wild-type and PPAR- $\beta/\delta^{-/-}$  mice, that such signals including TNF- $\alpha$  and IFN- $\gamma$ , induce keratinocyte differentiation was demonstrated. This cytokine-dependent cell differentiation pathway requires up-regulation of the PPAR- $\beta/\delta$  gene via the stress-associated

kinase cascade, which targets a receptor on the PPAR- $\beta/\delta$  promoter. In addition, pro-inflammatory cytokines, such as TNF- $\alpha$ , also initiate the production of endogenous PPAR- $\beta/\delta$  ligands, which are essential for PPAR- $\beta/\delta$  activation and action. Activated PPAR- $\beta/\delta$  regulates the expression of genes associated with apoptosis, resulting in an increased resistance of keratinocytes to cell death. This effect is also observed *in vivo* during wound healing after an injury. Recent, unpublished results from our laboratory has demonstrated that Manuka honey is capable of significantly increasing the expression of PPAR- $\beta/\delta$  in human monocytes. Although this result as yet remains unconfirmed in keratinocytes it is, nevertheless, early evidence that honey may induce expression of this important nuclear receptor involved in wound repair.

The provision of nutrients that enhance cell growth is another way that honey may stimulate healing in chronic wounds. A recent study has indicated that glucose enhanced the proliferation of human dermal fibroblasts *in vitro* (Hanet *et al*, 2004). Since glucose levels in exudates collected from chronic wounds are low (Tregrove *et al*, 1996), and honey contains approximately 33.5% glucose, it is possible that topical application of honey to chronic wounds helps to promote local cell growth.

There is one laboratory observation that helps to explain the documented clinical benefits of honey as a debriding agent. Proteolytic activity of honey from two different species of bees has been measured in bovine fibrinogen, with maximum activity at pH 5–6 (Oliveira *et al*, 2004). Previously, hydrolytic activity had not been associated with honey.

## Conclusion

The evidence that honey has an increasingly important role in wound repair will involve studies that investigate its ability to both modulate and interact with nuclear receptors such as PPAR- $\beta/\delta$ , and to release cytokines such as TNF- $\alpha$  and TGF- $\alpha_1$ . The complexity and variety of the components present in honey, requires a greater understanding of how they act, both individually and collectively, before deductions about specific mechanisms and clinical effects are possible.

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