PERSPECTIVE ARTICLE

# Honey: An immunomodulator in wound healing

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## ABSTRACT

Honey is a popular natural product that is used in the treatment of burns and a broad spectrum of injuries, in particular chronic wounds. The antibacterial potential of honey has been considered the exclusive criterion for its wound healing properties. The antibacterial activity of honey has recently been fully characterized in medical-grade honeys. Recently, the multifunctional immunomodulatory properties of honey have attracted much attention. The aim of this review is to provide closer insight into the potential immunomodulatory effects of honey in wound healing. Honey and its components are able to either stimulate or inhibit the release of certain cytokines (tumor necrosis factor-α, interleukin-1β, interleukin-6) from human monocytes and macrophages, depending on wound condition. Similarly, honey seems to either reduce or activate the production of reactive oxygen species from neutrophils, also depending on the wound microenvironment. The honey-induced activation of both types of immune cells could promote debridement of a wound and speed up the repair process. Similarly, human keratinocytes, fibroblasts, and endothelial cell responses (e.g., cell migration and proliferation, collagen matrix production, chemotaxis) are positively affected in the presence of honey; thus, honey may accelerate reepithelization and wound closure. The immunomodulatory activity of honey is highly complex because of the involvement of multiple quantitatively variable compounds among honeys of different origins. The identification of these individual compounds and their contributions to wound healing is crucial for a better understanding of the mechanisms behind honey-mediated healing of chronic wounds.

Honey has been used as a traditional medicine for centuries by different cultures for the treatment of various disorders including burns and chronic wounds. Honey offers broad spectrum antimicrobial properties and promotes rapid wound healing.[1] The antibacterial potential of honey has been considered the exclusive criterion for its wound healing properties. Therefore, the antibacterial activity of honey from different floral sources has been intensively studied over the past few decades. Recently, defensin1, one of the major antibacterial factors in honey, was shown to be a regular but quantitatively variable component of each honey.[2] One reason for the varying contents of defensin1 in different honeys seems to be constitutive but variable defensin1 expression in individual honeybees in bee populations.[3] It has also been found that some types of honey derived from specific floral sources become more potent than others because of the presence of phytochemicals with antibacterial properties.[4–6] These potent natural honeys, such as manuka (Medihoney, Comvita NZ Ltd., Te Puke, New Zealand) and RS honey (Bfactory Health Products B.V., Rhenen, The Netherlands) (honey with unknown origin used as a source for Revamil), are currently being used as medical-grade honeys in clinical applications. Medical-grade honey is being incorporated into sterile devices that are applied topically to wounds. However, honeys may also contain bee- or plant-derived substance(s) with immunomodulatory effects that can positively affect the wound healing process. Therefore, the antibacterial potential of honey may not be the sole criterion for selecting medical-grade honeys.

It has been assumed that the antibacterial action of honey has its main impact on the healing process of chronic wounds. Honey eliminates pathogens from wounds and provides an appropriate moist environment for proper wound healing. As the direct antimicrobial effects of honey were fully characterized in vitro, research has also focused on identifying the substances responsible for its immunomodulatory effects.[7,8]

| | |
|---|---|
| COX-2 | Cyclooxygenase-2 |
| IL | Interleukin |
| LPS | Lipopolysaccharide |
| MM6 | Mono Mac 6 |
| MMP-9 | Matrix metalloproteinase 9 |
| MRJP1 | Major royal jelly protein 1 |
| mRNA | Messenger ribonucleic acid |
| MW | Molecular weight |
| NO | Nitric oxide |
| ROS | Reactive oxygen species |
| TNF-α | Tumor necrosis factor-α |

**Table 1.** Immumodulatory compounds of various honey samples and their biological functions involved in honey-induced wound healing

| Specific factor(s) | Honey | Immunomodulatory activity | Reference |
|---|---|---|---|
| Arabinogalactans | Kanuka honey | Monocytes activation | Gannabathula et al.[18] |
| 261 MW component | Jungle honey | Neutrophils activation | Fukuda et al.[7] |
| 5.8 kDa component | Manuka honey | Monocytes activation | Tonks et al.[17] |
| MRJP1 | Acacia honey | Macrophages activation | Majtan et al.[20] |
| MRJP1 | Acacia honey | Keratinocytes activation | Majtan et al.[8] |
| Apigenin, Kaempferol | Honeydew honey | MMP-9 inhibition | Majtan et al.[55] |

MMP-9, matrix metalloproteinase 9; MRJP1, major royal jelly protein 1; MW, molecular weight.

Some promising candidates with immunomodulatory properties have been identified in honey (Table 1), but further research is necessary to prove these immunomodulatory properties.

The aim of this work is to review the immunomodulatory effects of natural honey on immune and cutaneous cells that participate in the wound healing process and to elucidate the different mechanisms of honey-induced immunomodulation.

## HONEY AND CYTOKINE PRODUCTION

Besides providing a structural barrier, the skin contains several types of immune cells that can be activated by skin damage. One of the most important groups of immune cells involved in wound healing are macrophages, which exhibit different immunological functions in the skin, including phagocytosis and antigen presentation. Tissue macrophages are cells derived from peripheral blood monocytes. In injured tissue, monocytes migrate through the vessel wall; they release enzymes that fragment extracellular matrix proteins, creating space for monocytes to migrate to the wound bed. Macrophages can be activated either classically (by lipopolysaccharide [LPS] and interferon-γ) or alternatively (by interleukin [IL]-4 and IL-13).[9,10] LPS-stimulated macrophages are capable of synthesizing and secreting inflammatory mediators, including tumor necrosis factor-α (TNF-α), nitric oxide (NO), and IL-6. IL-4-activated macrophages play important roles in wound healing and angiogenesis.[10]

In addition to the above-mentioned properties, macrophages produce many other cytokines and growth factors that stimulate new capillary growth, collagen synthesis, and fibrosis.[11]

In recent years, several groups have examined honey and/or its individual components in order to elucidate its wound healing properties. Macrophages/monocytes are a suitable model for monitoring the immunomodulatory activity of novel potential immunomodulators. Tonks and coworkers suggested that the wound healing effect of honey may be partly related to the release of proinflammatory cytokines from surrounding cells, mainly monocytes and macrophages.[12,13] An immunomodulatory effect was showed by cytokine release from the monocytic cell line Mono Mac 6 (MM6) and human peripheral monocytes after incubation with 1% (w/v) honey. Several natural honeys were used in this study, including manuka and jelly bush honey. All types of honey induced or stimulated the release of TNF-α, IL-1β, and IL-6 from MM6 cells and peripheral blood monocytes when compared with the syrup control (artificial honey) and untreated cells. The MM6 cells treated with jelly bush honey showed a significantly higher above-mentioned cytokines release than cells treated with manuka or the other natural honeys. The authors of the study also claimed that the concentration of endotoxins in all natural honeys (from 56 to 690 pg/mL) is negligible, and that stimulation of MM6 cells is independent of endotoxins. However, it is important to note that MM6 cells are very sensitive to endotoxins,[14] and it is very likely that the endotoxin content of honey could be responsible for its stimulatory effect. Endotoxins possess special characteristics. They are, to a large extent, heat stable, and their activity can be abrogated by the antibiotic polymyxin B.[15] It has been shown that MM6 cells responded to an endotoxin with a detection limit as low as 3.1 pg/mL,[16] and that robust release of IL-6 occurred when they were stimulated with 100 pg/mL endotoxin.

In a recent study, Timm et al. (2008) investigated the effect of four different honeys including manuka honey on the release of important proinflammatory cytokine (IL-6) from MM6 cells.[14] Similar to previous studies,[12,13] natural honeys induced maximal release of IL-6 after 18 hours of treatment. They reported that the substances in honey responsible for its immunomodulatory activity are (1) heat stable; (2) retained in the high molecular weight (MW) fraction (>20 kDa); and that (3) their activity was abrogated when the honey was incubated with polymyxin B, an inhibitor of endotoxin activity. All of these characteristics are in concordance with the properties of endotoxins. In contrast to these findings, Tonks et al. demonstrated that heat treatment caused a significant reduction in the ability of honey to stimulate cytokine production in MM6 cells.[17] Moreover, the cytokine-stimulatory effect of honey was assessed in the presence of polymyxin B. Similarly, the ability of New Zealand honeys to release TNF-α from the monocytic cell lines THP-1 and U937 has recently been characterized.[18] The immunomodulatory activity of all the honeys was associated with a high MW (>30 kDa) component that was partially heat labile and inhibitable with polymyxin B.[18]

A number of peptides and proteins from natural sources are known for their nonspecific immunostimulatory responses.[19] Peptide and protein immunomodulators, in general, generate a physiological response in target cells via their specific receptors. Glycosylated proteins are known to induce TNF-α secretion from macrophages, and this cytokine is known to induce wound repair mechanisms. We have previously shown that a natural acacia honey is able to stimulate TNF-α secretion from murine macrophages, whereas deproteinized honey

has no effect on the release of TNF-α.[20] This suggests that the protein content of honey, primarily the 55 kDa glycoprotein major royal jelly protein 1 (MRJP1), which is the dominant protein in royal jelly[21] as well as in honey,[22] might be responsible for the immunomodulatory effects of honey. Our previous results also showed that the production of TNF-α from murine macrophages is actually increased after limited proteolytic digestion. We found that the N-terminal region of recombinant MRJP1 elicited marked release of TNF-α. The endotoxin content of acacia honey or of native and recombinant MRJP1 samples was not determined in our study. It is very likely that samples of purified MRJP1 contain endotoxins at a sufficient level to stimulate the release of TNF-α from murine macrophages. Therefore, we can assume that endotoxins in honey may play an important role in the activation of monocytes and/or macrophages depending on the individual honey. On the other hand, it has been reported that MRJP1, at concentration of 25 μg/mL, increased the level of TNF-α messenger ribonucleic acid (mRNA) expression twofold in primary cultures of epidermal keratinocytes.[8] Similarly, an upward trend in mRNA expression of IL-1β and transforming growth factor-β was observed following treatment with MRJP1 in human keratinocytes.

Another promising immunostimulatory protein identified in honey belongs to the group of type II arabinogalactan proteins, with an MW of about 110 kDa. Type II arabinogalactan proteins from a range of sources have been shown to have immunomodulatory properties.[23] They are able to stimulate the release of TNF-α from monocytic cell lines THP-1 and U937.[18]

Although honey is a natural product and rich in various phytochemical and bee-derived compounds that may possess immunomodulatory activities, some researchers have postulated that the immunomodulatory effects of honey could be because of its endotoxin content.[14] Sterilization of honey using gamma irradiation effectively eliminates bacterial spores and vegetative forms of any bacteria present; however, bacterial endotoxins may still remain present. Bacterial endotoxins (LPSs), major components of the outer membrane of Gram-negative bacteria, are complex glycolipids composed of a hydrophilic polysaccharide moiety and a hydrophobic domain known as lipid A. Endotoxins activate macrophages to produce proinflammatory cytokines. The production of these cytokines is tightly regulated as excessive production leads to amplified inflammatory responses and devastating illness characteristic of severe septic shock.[24]

## HONEY AND REACTIVE OXYGEN SPECIES (ROSs)

Many studies suggest that honey rapidly eradicates infection with no adverse effects, reduces inflammation, swelling, pain, and odor, and also stimulates the wound healing process.[25–29] Research supporting positive clinical observations has mainly focused on the anti-inflammatory and antioxidant properties of honey.

Chronic wounds are considered to be highly oxidizing environments owing to the release of ROS from infiltrating neutrophils and macrophages. ROSs are thought to possess certain beneficial antimicrobial properties against invading bacteria;[30] prolonged exposure to elevated levels of ROS causes cell damage and may inhibit the healing of both acute and chronic wounds.

Therefore, one way to interrupt chronic inflammatory cycle is to remove ROS with antioxidants, and honey is known to contain antioxidants that scavenge free radicals.[31,32] Various components of honey contribute to its antioxidant properties, including flavonoids, phenolic acids, catalase, peroxidise, ascorbic acid, and carotenoids, and products of the Maillard reaction.[33] The quantity of these components varies according to the floral and geographical origin of each type of honey.[34–37] Several studies have shown that phenolic compounds in honey are partially responsible for its antibacterial and antioxidant activities.[36,38–40] It has been shown that ROSs mediate TNF-α-induced cytotoxicity, which can be blocked by specific free radical scavengers (e.g., flavonoids).[41,42] In fact, Habtermariam[43] demonstrated that phenolic compounds, such as caffeic acid, effectively inhibit TNF-α-induced cytotoxicity in L929 cells. In a very recent study,[44] a honey methanol extract and a honey ethyl acetate extract were tested in vitro for their effect on NO production in the endotoxin- and IFN-γ-stimulated murine macrophage cell line RAW264.7. It was shown that both honey extracts were capable of inhibiting NO production in the macrophages. The concentration of NO was inhibited in a dose-dependent manner in the presence of the honey extracts. The honey ethyl acetate extract exhibited greater activity than the honey methanol extract. However, the methanol extract contained a higher concentration of phenolic compounds, where the majority of the phenolics were ellagic, gallic, and ferulic acids, myricetin, chlorogenic acid, and caffeic acid. Similarly, Woo et al.[45] found that chrysin, a natural flavonoid found in many plant extracts, honey, and propolis[46,47] inhibited cyclooxygenase-2 (COX-2) gene expression in LPS-stimulated cultured macrophages, and this effect was mediated through inhibition of the binding activity of nuclear factor IL-6. The fact that nuclear factor IL-6 is negatively regulated by chrysin is important because this transcription factor plays a critical role in the regulation of a variety of genes involved in inflammatory responses.

Another study, by Ahmad et al., supports the hypothesis that honey exhibits its anti-inflammatory activity through inhibition of activated macrophages.[48] They found that honey treatment of rodent macrophages activated by bovine thrombin resulted in effective suppression of oxidative respiratory bursts. Interestingly, all honey samples from different origins showed effective suppression.

Taken together, these findings are contradictory, and it is difficult to distinguish which molecule(s) in honey is fully responsible for its immunomodulatory effect. It is important to carry out further detailed research in order to explain the immunomodulatory effect of honey on macrophages/monocytes.

Persistent neutrophil infiltration and release of ROS by neutrophils contribute to the pathophysiology of chronic wounds. A decrease in neutrophil superoxide production by honeys has recently been reported.[31,49,50] An antioxidant activity of honeys was attributed to inhibition of ROS formation, either by inhibiting the respiratory burst of neutrophils or by direct ROS scavenging.[32] Interestingly, a dose-dependent reduction in human neutrophils' superoxide production by honeys did not correlate with the levels of known honey-based phenolic compounds, which are well-known free radical scavengers.[50] This observation indicates that the antioxidant activity of honey is likely caused by inhibition of neutrophils' respiratory burst.

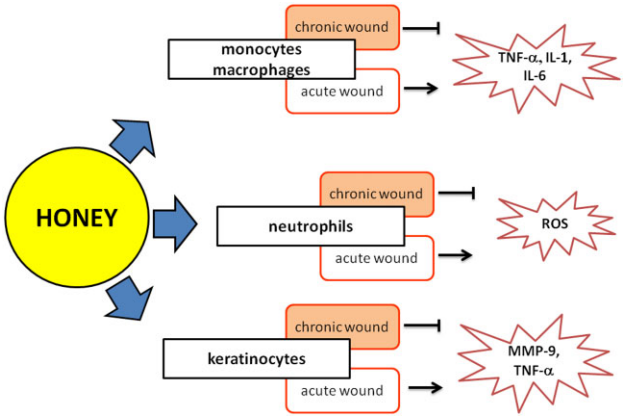


**Figure 1.** The immunomodulatory action of honey on immune and cutaneous cells involved in wound healing. Honey is able to either stimulate or inhibit the release of certain factors (cytokines, MMP-9, ROS) from immune and cutaneous cells depending on wound condition. Honey induces secretion of proinflammatory cytokines and MMP-9 during the inflammatory and proliferative wound healing phase, respectively. On the other hand, when the wound inflammation is uncontrolled, honey abrogates prolonged wound inflammation and reduces the elevated levels of proinflammatory cytokines, ROS, and MMP-9. IL, interleukin; MMP-9, matrix metalloproteinase-9; ROS, reactive oxygen species; TNF-α, tumor necrosis factor α.

In a very recent study, a compound with an MW of 261 Da isolated from jungle honey was found to elicit chemotactic activity in neutrophils.[7] The authors of this study also investigated the mechanism of the antitumor activity of jungle honey, which seemed to be related to the production of ROS by activated neutrophils. The jungle honey was injected into tumor tissues in mice, and many neutrophils infiltrated necrotic areas in the tumor and produced ROS. The incidence and mean weight of the tumors decreased in jungle honey-injected mice.

Taking these results together, honey seems to either reduce or activate the production of ROS from neutrophils, depending upon the microenvironment (Figure 1).

## ANTI-INFLAMMATORY ACTIONS OF HONEY

Reduced inflammation observed in the clinic following the application of honey is supported by histological evidence of reduced numbers of inflammatory cells present in wound tissue.[51] Inflammation is a nonspecific response of mammalian tissue to a variety of hostile agents.[52] There are many mediators of inflammation, such as endotoxins, some cytokines, and NO. Therefore, the inhibition of inflammatory mediators is one of the important steps in controlling inflammation.

Honey exhibits potent multiple anti-inflammatory effects. Clinically, there have been numerous observations reported of honey reducing edema and exudate, minimizing scarring and having a soothing effect when applied to inflamed wounds and burns (reviewed in Molan[53]). The anti-inflammatory effect of honey may be explained by several mechanisms of action: (1) inhibition of the classical complement pathway;[31] (2) inhibition of ROS formation;[31] (3) inhibition of leukocyte infiltration;[50] and (4) inhibition of COX-2 and inducible NO synthase expression.[54] Finally, the inhibition of matrix metalloproteinase 9 (MMP-9), a major protease responsible for the degradation of matrix and cell growth-promoting agents in chronic wound fluids, in human keratinocytes has been reported very recently[55] and represents another novel anti-inflammatory mechanism of honey action.

In a very recent study, we found that acacia honey at a concentration of 1% (w/v) significantly enhanced the expression of MMP-9 mRNA in primary cultures of human keratinocytes.[8] Furthermore, incubation of human skin fragments with honey for 24 hours was associated with increased expression of MMP-9 protein in the epidermis near the basement membrane. Subsequently, we also found a decrease in the relative amount of collagen type IV in the basement membrane and around the blood vessels following incubation of the skin with honey for 24 hours. These results appear contradictory to the results presented in our very recent study[55] where honey inhibited TNF-α-induced MMP-9 expression. Therefore, we assume that honey can act as an immunomodulator with both proinflammatory and anti-inflammatory properties (Figure 1). We speculate that honey stimulates the production of inflammatory cytokines and MMP-9 from keratinocytes when a low level of an inflammatory/stimulatory mediator is present. On the other hand, if an environment is infected and inflammation is in progress, honey suppresses the production of inflammatory cytokines and MMP-9. This hypothesis is very promising and could result in new therapeutic advantages for the treatment of skin inflammation in the future.

To date, the components including phenolic compounds and flavonoids responsible for the anti-inflammatory honey in vitro activities have been partially identified. However, it is not clear whether these components within honey exhibit anti-inflammatory activities in vivo.

## CONCLUSION

Honey, at medical-grade level, is a high-quality wound care product, as supported by the sheer number of papers in the recent scientific literature. It has been found to be particularly effective where standard wound care is limited or unsuccessful. However, some wound-care professionals are still skeptical about the benefits of honey in wound care. As the antibacterial action of honey is well characterized, there is a need to fully elucidate the compounds/mechanisms responsible for honey's immunomodulatory and anti-inflammatory properties in order to support a positive clinical outcome of using honey in wound management.

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