How photons modulate wound healing via the immune system

Mary Dyson
King's College London (KCL), University of London, Guy's Hospital Campus, London SE1 9RT, UK.

ABSTRACT

The immune system is a diverse group of cells that recognize and attack foreign substances, pathogenic organisms and cancer cells. It also produces inflammation, an essential component of the wound healing process and, following the resolution of inflammation, plays a crucial role in the control of granulation tissue formation. Granulation tissue is the precursor of scar tissue. Injured skin and mucous membranes generally heal rapidly. However, some wounds are either slow to heal or fail to heal while in others overgrowth of scar tissue occurs, resulting in the production of either hypertophic or keloid scars. The modulation of wound healing in such conditions is clinically important and may even be vital. Evidence will be presented that phototherapy can modulate wound healing, and that changes induced in the immune system, in particular the secretion of soluble protein mediators including cytokines, may be involved in this modulation. The immune system has peripheral and deep components. The former, being located mainly in the skin and mucous membranes, are readily accessible to photons, which can affect them directly. The components of the immune system are linked by lymphatic vessels and blood vessels, which include many capillaries located in the sub-epithelial connective tissues of the skin and mucous membranes. The superficial location of these capillaries provides the immune cells and molecules in transit through them with ready access to photons. When these cells and molecules, some modified by exposure to photons, reach susceptible cells such as lymphocytes in the deeper parts of the immune system and cells of injured tissues, they can modify their activity. In addition to having direct effects on peripheral cells, photons can thus also produce indirect effects on cells too distant for the photons to reach them. For example, cytokines released from peripheral macrophages in response to the direct action of photons can be transported to and affect other cells, including fibroblasts of injured tissues, that have not been exposed to photons. It is therefore possible for injuries other than those directly exposed to phototherapy to be affected by it indirectly.

Keywords: Phototherapy, immune system, inflammation , proliferation, remodeling, delayed wound healing , cytokines, growth factors

1. INTRODUCTION

The immune system, part of the haemolymphoid system [1], plays a vital role in the response of the body to pathogens, cancer and injury [2]. The key molecular components of the immune system are antibodies and soluble protein mediators (SPMs) such as cytokines and growth factors.

The main cellular components of the immune system are lymphocytes and macrophages. These are located either in peripheral tissues such as the epidermis and dermis of the skin and the epithelium and lamina propria of mucous membranes or in deeper organs such as the deep lymph nodes and spleen. All the components of the immune system are linked by blood vessels and lymphatic vessels, via which immune cells and the molecules they secrete are carried around the body. SPMs released from peripheral immune cells in response to the direct action of photons can be transported to and affect cells that have not been exposed to photons. Injuries other than those directly exposed to photons can be affected by them indirectly.

Following a brief description of the functional anatomy of the immune system and of the phases of wound healing, the effects of phototherapy on the components of the immune system are through them on wound healing are reviewed.

2. FUNCTIONAL ANATOMY OF THE IMMUNE SYSTEM

The body has *first, second and third lines of defense*. If pathogens and other foreign, non-self, materials penetrate the body's first line of defense, the outer epithelium of the skin and mucous membranes that normally prevents their entry, the immune system is activated, its innate component forming the body's second line of defense and its adaptive component the body's third line of defense.

2.1 Innate immune system

The innate immune system, the body's *second line of defense*, is activated if the first line of defense fails. Its cells, which include Langerhans cells, are mainly located in the skin associated lymphoid tissue (SALT)[3] and mucous membrane associated lymphoid tissue (MALT). Their superficial location renders them accessible to photons during phototherapy. Other immune cells, the natural killer (NK) cells, patrol the body in the blood and lymph, lysing cancer cells and virus-infected cells. The response of the innate immune system is non-specific and immediate. It is enhanced by cytotoxins secreted by the NK cells. During it neutrophils, macrophages, NK cells, T lymphocytes and antimicrobial proteins inhibit the spread of the invading substances. SPMs released locally recruit immune cells to the infected region and promote tissue repair. If the second line of defense fails, the *third line of defense*, the adaptive immune system, is activated.

SPMs consist of 3 groups:

1. CHEMOKINES, for example fractalkine, are chemotactic molecules that attract and activate inflammatory cells

2. CYTOKINES, for example interleukins, are molecules that regulate division and differentiation of immune (inflammatory) cells

3. GROWTH FACTORS, for example platelet derived growth factor (PDGF), are molecules that stimulate division of both immune and non-immune (non-inflammatory) cells.

Immune or inflammatory cells include Langerhans cells, neutrophils, natural killer cells, monocytes, macrophages, T & B lymphocytes, plasma cells and mast cells. All play significant roles during the inflammatory and proliferative phases of wound healing. Non-immune or non-inflammatory cells that are of importance during wound healing include epithelial cells, endothelial cells, fibroblasts and myofibroblasts.

Photons can be absorbed not only by the superficially-located immune cells of the SALT and MALT and but also by immune cells in transit through the superficially-located blood and lymph capillaries of the skin and mucous membranes. Phototherapy can have a direct effect on the secretion of SPMs by these cells. By doing so it can accelerate the resolution of inflammation and thereby accelerate repair if this is delayed [4,5]. The deeper cells of the immune system and also non-immune cells of injured tissues can be affected indirectly by SPMs released from peripherally-located cells that have absorbed photons. Cells of injured tissues are more sensitive to phototherapy that cells of intact tissues[6]. Phototherapy thus has both local and systemic effects. The secretion of different SPMs may assist chronic wounds to heal by allowing them to progress from inflammation to the proliferative phase of wound healing when granulation tissue is formed and re-epithelialization occurs. Because of these systemic effects the use of phototherapy to treat one wound of a patient may lead to improvements not only in this wound but in the patient's other wounds.

2.2 Adaptive immune system

The adaptive immune system, the body's *third line of* defense, is activated if the second line of defense fails. Its cells include macrophages, T lymphocytes and B lymphocytes, located in peripheral SALT and MALT, in more deeply positioned lymphoid organs such as deep lymph nodes and the spleen, and being transported in the blood and lymph. The superficial location of the SALT and MALT and of immune cells in transit through the superficial capillaries allows them to absorb photons and be affected by them directly. The deeper lymphoid organs can be affected indirectly. The response of the adaptive immune system is antigen-specific. Antigens are substances that bind to specific immune receptors and elicit an immune response, such as the production of specific antibodies by B lymphocytes and plasma cells. The adaptive immune system has cell-mediated and humoral components. Exposure to antigens produces an immunological memory, improving the ability of the body to respond to and recognize these antigens if they are encountered again.

2.3 Link between cutaneous nerves and SALT

Cutaneous contact hypersensitivity (CH) reactions are closely correlated with Langerhans cells (LC), macrophages that arise from stem cells in the bone marrow and migrate into the epidermis. Also known as epidermal dendritic cells they help to activate the immune system by presenting antigens to lymphocytes. LCs may be linked synaptically to cutaneous nerve termini containing calcitonin gene-related peptide (CGRP), suggesting that there is a link between innervation and immune responses in the skin. It has been proposed that 'cutaneous nerves dictate whether antigen applied to the skin will lead to sensitivity or tolerance'[2], linking the nervous system to the immune system. There is evidence that laser therapy can affect mast cell degranulation[6] resulting in activation of pain fibres. Nerve conduction[7] is also affected by phototherapy, supporting the hypothesis that it may affect the immune system via the nervous system.

3. WOUND HEALING

3.1 Outline

Wound healing consists of a closely regulated cascade of events that follow injury and that in skin normally result in the regeneration of the epidermis and the replacement of the damaged dermis with scar tissue. The events can be grouped into the sequential and overlapping phases of *inflammation*, *proliferation,* and *remodeling.* If the dermis is damaged, hemostasis is the initial major component of inflammation, following which debris and damaged tissue are removed from the wound site by neutrophils and macrophages. Antigens are also detected and presented to T-lymphocytes by macrophages such as Langerhans cells. During proliferation, angiogenesis and the formation of matrix rich in type III collagen results in the production of granulation tissue over which the epidermis migrates and regenerates. Myofibroblasts which develop in the granulation tissue produce wound contraction, reducing the size of the wound. During remodeling, the granulation tissue is gradually transformed into less vascular, less cellular and more collagenous scar tissue which replaces the injured dermis. Much of the type III collagen is replaced by stronger type I collagen arranged in wider fiber bundles, increasing the tensile strength of the scar tissue although this remains weaker than uninjured dermis.

3.2 Regulation of wound healing

For wound healing to be successful, the multitude of events comprising it must be spatially and temporally regulated. This regulation is highly dependent on intercellular communication. Soluble protein mediators (SPMs), consisting of chemokines, cytokines and growth factors, are among the main substances involved in this communication: the other substances include hormones, neurotransmitters, receptors, protease and protease inhibitors[8]. Many of the SPMs are produced by immune cells, eg neutrophils, macrophages and lymphocytes. Following SPM synthesis and secretion, the SPMs diffuse or are transported in blood and lymph vessels to target cells involved in the healing process. They bind to specific receptor sites on the target cell surface. Binding triggers target cell activation, the activity depending on the target cell type. For example, myofibroblasts will contract, fibroblasts will (depending on their stage of differentiation) either proliferate or secrete matrix materials.

SPM actions during wound healing include the following:

1. Action: Initiation of inflammation. SPMs : Il-1, TNF, etc.

2. Action: Cell recruitment to wound bed. SPMs: PAF, Il-1, Il-3, Il-6, TNF, etc.

3. Action: Debris removal. SPMs : Il-1, Il-2, Il-4, Il-5, Il-6, TNF, etc.

4. Action: Promotion of proliferative phase of healing. SPMs: FGF, PDGF, TGF-b, Il-1, Il-6, TNF etc

Key: Il = Interleukin; TNF = Tumor necrosis factor, PAF = Platelet activating factor, FGF = Fibroblast growth factor, PDGF = Platelet derived growth factor, TGF-b = Transforming growth factor-beta.

4. EXPERIMENTAL EVIDENCE OF ALTERED IMMUNE RESPONSE FOLLOWING PHOTOTHERAPY

4.1 Normalization of immunological activity

A study of the effects of magnetic and laser therapy on the healing of fractures and blood levels of T and B lymphocytes in dogs has confirmed that laser therapy can stimulate fracture repair and has demonstrated a normalization of the immunological activity[9].

4.2 Suppression of immune system

Phototherapy delivered transcutaneously at high energy density (1,589 J/cm^2) over spinal cord injuries in rats has been shown to significantly suppress immune cell activation and cytokine/chemokine expression within the spinal cord[10]. This suppression should reduce the development of collagen at the site of the injury and thus facilitate the growth of nerve fibers across the spinal cord defect. Treatment was with an 810nm, 150 mW diode laser.

4.3 Cutaneous immunological activation

A low-fluence pulsed dye laser operating at 585 nm, reported to be effective in the treatment of acne and atopic dermatitis and as a wrinkle reducing device, has been shown to produce direct cutaneous immunological activation in 8 human volunteers. 3 hours after laser treatment neutrophils, monocytes and mast cells were detected in the dermis. These acute inflammatory changes were also observed 1 week after treatment. Four weeks after treatment many lymphocytes were observed. One week after laser treatment all the subjects were positive for IL-2 and IL-4 mRNA, the level of IL-4 mRNA being larger than that of IL-2 mRNA[11].

5. EXAMPLES OF EFFECTS OF PHOTOTHERAPY ON IMMUNE CELLS

5.1 Neutrophils

Low-level laser therapy (LLLT) attenuates reactive oxygen species (ROS) production by human neutrophils and may therefore be effective in the treatment of inflammation[12]. The attenuating effect was larger in the neutrophils of smokers than of non-smokers. The authors suggest that LLLT may improve wound healing in smokers via its effects on inflammation. The laser device used was a diode laser, 830 nm, continuous wave, applied with an energy density of 150 mW/cm^2.

5.2 Mononuclear cells

The proliferation of peripheral blood mononuclear cells can be enhanced by treatment in vivo with 632.8 nm low energy He-Ne, although this is less effective than the mitogenic stimulator phytohemaglutinin (PHA)[13]. Should this light-induced increase in proliferation also occur in vivo it could increase the supply of monocytes to injured tissues where they differentiate into macrophages.

5.3 Macrophages

A. In addition to phagocytosing debris at injury sites, macrophages also secrete cytokines that control the progression from inflammation to the proliferative phase of tissue repair[2,8]. Phototherapy has been shown to modify the uptake of calcium ions by macrophages in vitro[14], this modification of membrane permeability triggering cell activity. The increase in uptake of calcium ions by macrophages exposed to red light and infrared radiation has been shown to be both wavelength and energy density dependent. Of the wavelengths tested, 660, 820 and 870 nm were effective but 880 nm was not. Of the energy densities tested, 4 and 8 J/cm^2 were effective, but 2 and 19 J/cm^2 were not. This indicates that there is a therapeutic window of effectiveness; if the energy level supplied is either too low or too high the therapeutic response required will not be achieved. The wavelengths and energy densities that increased calcium uptake also stimulated secretion of growth factors into the medium in which the macrophages had been cultured. When this medium was added to cultures of fibroblasts that had not been exposed to phototherapy their division was stimulated, demonstrating that phototherapy can affect cells indirectly[15] via SPMs.

B. A study of the effects of low-intensity laser radiation (632.8 nm, 0.2 mW/cm^2) on murine macrophages has shown a significant stimulation in their production of cellular tumor necrosis factor-alpha (TNF-α) at exposures of from 5 to 180 s. Furthermore, TNF-α and IL-6 production were enhanced after the minimum exposure (5s), whereas prolonged exposure (60 and 180 s) did not induce changes in IL-6 production, indicating the importance of the energy level, and possibly treatment time, in activation of specific aspects of cellular metabolism[16].

5.4 Lymphocytes

A. The proliferation of human T-lymphocytes can be modulated in vitro by exposure to phototherapy of 820 nm wavelength at 50 mW and a pulsing frequency of 5 kHz. The energy densities used ranged from 1.2 to 13.2 J/cm^2. It was found that in actively proliferating cells treated with the mitogen PHA, proliferation was reduced by laser treatment throughout the range of energy densities used. In contrast, in non-mitogen treated T-lymphocytes, energy densities of 1.2 and 3.6 J/cm^2 stimulated their proliferation whereas higher energy densities (10.8 and 13.2 J/cm^2) were inhibitory[17]. These results support the hypothesis that the response of cells depends, in part, on their metabolic status.

B. Low-intensity laser radiation (632.8 nm, 0.2 mW/cm^2) at exposures of 60 or 180 s decreased IL-6 production by murine splenic lymphocytes in vitro[16]. When considered with the findings in the other immune cells reported above and in vivo (4.1- 4.3) a trend emerges: short duration irradiation tends to induce immuno-activation, whereas prolongation of exposure tends to induce immunosuppression.

6. CLINICAL RELEVANCE TO WOUND HEALING

Phototherapy has been used for many decades to treat the wounds of patients in whom healing is delayed[18]. It is suggested that treatment of the intact skin around chronic wounds may, provided that the correct parameters are used, activate immune cells of the SALT. This will increase the efficiency with which pathogens and debris are removed and stimulate the release of cytokines of value in the proliferative phase of repair. Furthermore latent SPMs such as transforming growth factor–beta 1 (TGF-ß1), of crucial importance in wound healing, can be activated by phototherapy[19]. In addition to exposing SALT to phototherapy, irradiation of peripheral lymph nodes could also be of

value in that more immune cells will be exposed to the beneficial effects of phototherapy. Immune cells from these nodes will enter the lymphatics and be transported to the wounds where they and the cytokines they secrete can assist in the healing process[20].

It is possible that variation in the treatment parameters used may determine which SPMs are secreted. Different mediators are necessary for different activities during wound healing, including the initiation of inflammation, the recruitment of inflammatory and non-inflammatory cells to the wound bed, debris removal by neutrophils and macrophages, and the induction of granulation tissue formation. Chronic wounds may be trapped in the inflammatory phase of healing; compared with healing wounds, they have more inflammatory cytokines, higher protease activity, lower mitogenic activity and contain fewer mitotically competent cells[8]. Selection of appropriate treatment parameters may move them on to the proliferative phase of healing. What these parameters are remains to be determined. Antibody array screening allows the rapid monitoring of the induction of different SPMs[21]. Selection of the best parameters could optimize the treatment of chronic wounds with phototherapy, helping improve the quality of life of millions of people world wide. Photon-induced effects on hypertophic and keloid scars involving the immune system require investigation.

7. CONCLUSIONS

Cells of the immune system initiate acute inflammation, an essential part of the healing process. The peripheral components of the immune system such as the Langerhans cells of the epidermis are readily accessible to photons and can be affected by them directly, triggering the release of a variety of SPMs which orchestrate the sequential events of the inflammatory, proliferative and remodeling phases of wound healing. These SPMs can either diffuse or be transported by blood and lymph vessels to the other parts of the immune system and to distant injured tissue where they can initiate reparative changes, thus amplifying the direct effects of the superficially absorbed photons. Cells can therefore be affected indirectly by photons without the need to absorb them. Photon-induced changes in peripherally located nerve fibers and in the endocrine system can also modulate wound healing either directly or indirectly. There is some evidence that exposure of immune cells different parameters of phototherapy can alter the types of SPMs produced. It may therefore be possible to select which treatments should be used to improve healing where it is defective. Further research on the effects of different parameters on SPM production by immune cells is indicated.

Acknowledgements

It is with great pleasure that I acknowledge and thank my many colleagues at Guy's Hospital whose research is referred to in this paper. They include Drs Atif Aaiby, Peter Bolton, Lucy Ghali, Salah El Sayed, Wil Harvey and Steve Young.

References

[1] Bannister L (1995) "Haemolymphoid system". *Gray's Anatomy*, Edited by Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, Ferguson MJW, 1399-1450. Churchill Livingstone, Edinburgh.

[2] Streilen JW, Alard P, Niizeki H. (1999) "A new concept of skin-associated lymphoid tissue (SALT): UVB light impaired cutaneous immunity reveals a prominent role for cutaneous nerves". The Keio Journal of Medicine. PMID: 10206015

[3] Martin P, Leibovich S (2005) "Inflammatory cells during wound repair: the good, the bad and the ugly". Trends Cell Biol 15(11):599-606.

[4] Dyson M, Young S (1986) "The effects of laser therapy on wound contraction and cellularity". Laser Med. Sci. 1:125-130.

[5] Dyson M (2007) "Adjuvant therapies: ultrasound, laser therapy, electrical stimulation, hyperbaric oxygen and Vacuum-assisted closure". *Leg Ulcers: a problem-based learning approach*. Edited by Morison MJ, Moffatt CJ, Franks PJ, 429-451.Mosby Elsevier, Edinburgh.

[6] El Sayed S, Dyson M (1990) A comparison of the effect of multi-wavelength light produced by a cluster of semiconductor diodes and each individual diode on mast cell number and degranulation in intact and injured skin". Laser Surg Med 10:559-568

[7] Vinck E, Coorevits P, Cagnie B, De Muynck M, Vanderstraeten G, Cambier D (2005) "Evidence of changes in sural nerve conduction mediated by light emitting diode irradiation". Lasers Med Sci 20(1):35-40.

[8]Ovington LG, Schultz GS (2004) The physiology of wound healing. *Chronic Wound Care: a problem-based learning approach*. Edited by Morison MJ, Ovington LG, Wilkie K, 83-100. Mosby Elsevier, Edinburgh.

[9] Balbekov IM, Khanaplyaev UK (2001) "Healing of bone fractures of rat shin and some immunological indices during magnetic and laser therapy and osteosynthesis by the Ilizarov method". Bull Exp Biol Med 131(4):399-402

[10] Byrnes KR, Waynant RW, Ilev IK, Barna L, Smith K, Heckert R, Gerst H, Anders JJ (2005) "Light promotes regeneration and functional recovery and alters the immune response after spinal cord injury". Lasers Surg Med 36(3):171-185.

[11] Omi T, Kawana S, Sato S, Takezaki S, Honda M, Igarashi T, Hankins RW, Bjerring P, Thestrup-Pedersen K (2005) "Cutaneous immunological activation elicited by a low-fluence pulsed dye laser". Br J Dermatology 153 Suppl 2:57-62.

[12] Fujimaki, Shimoyama T, Liu Q, Nakaji S, Sugawara K (2003) "Low-level laser irradiation attenuates production of reactive oxygen species by human neutrophils". J Clin Laser Med Surg 21(4):165-170.

[13] Gulsoy M, Ozer GH, Bozkulak O, Tabakoglu HO, Aktas E, Deniz G, Ertan C (2006) "The biological effects of 632.8-nm low energy He-Ne laser on peripheral blood mononuclear cells in vitro". J Photochem Photobiol B 8293):199-202.

[14] Young SR, Dyson M, Bolton P (1990) "Effect of light on calcium uptake by macrophages". Laser Therapy 2:53-57.

[15] Young S, Bolton P, Dyson M, Harvey W, Diamantolpoulos C (1989) "Macrophage responsiveness to light therapy. Lasers Surg Med 9:497-505.

[16] Novoselova EG, Cherenkov DA, Glushkova OV, Novoselova TV (2006) "Effect of low-intensity laser radiation (632.8 nm) on immune cells isolated from mice". Biofizika 51(3):509-518.

[17] Agaiby A, Ghali L, Dyson M (1998) "Laser modulation of T-lymphocyte proliferation in vitro. Laser Therapy, 10:153-158.

[18] Mester E, Mester AF, Mester A (1985) "The biomedical effect of laser application". Lasers Surg Med 5:31-39.

[19] Arany PV (2007) Personal communication.

[20] Dyson M (2008) "How phototherapy affects the immune system". In "Mechanisms for Low-Light Therapy", edited by Hamblin MR, Waynant RW, Anders J. Proc. SPIE 6846: 68605-1 – 68605-10.

[21] Chang DT, Jones JA, Meyerson H et al (2008) " Lymphocyte/macrophage interactions: Biomedical surface dependent cytokine, chemokine and matrix protein production". J Biomed Mater Res A (Epub ahead of print, accessed via PubMed).