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## Review

# Keloid pathophysiology: fibroblast or inflammatory disorders?

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## ABSTRACT

**Background:** Keloids are defined as a benign dermal fibroproliferative disorder with no malignant potential. They tend to occur following trivial trauma or any form of trauma in genetically predisposed individuals. Keloids are known to grow beyond the margins of the wound and are common in certain body parts. The pathophysiology of keloid remains unclear, and fibroblasts have been presumed to be the main cells involved in keloid formation. Understanding the mechanism(s) of keloid formation could be critical in the identification of novel therapeutic regimen for the treatment of the keloids.

**Objective:** To review the pertinent literature and provide updated information on keloid pathophysiology.

**Data Source:** A Medline PubMed literature search was performed for relevant publications.

**Results:** A total of 66 publications were retrieved, with relevant publications on the etiology and pathogenesis as well as experimental studies on keloids. All articles were critically analyzed, and all the findings were edited and summarized

**Conclusion:** There is still no consensus as on what is the main driving cell to keloid formation. One may, however, hypothesize that keloid formation could be a result of an abnormal response to tissue injury, hence resulting in an exaggerated inflammatory state characterized by entry of excessive inflammatory cells into the

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wound, including macrophages, lymphocytes, and mast cells. These cells seem to release cytokines including transforming growth factor  $\beta$ 1 that stimulate fibroblasts to synthesize excess collagen, which is a hallmark of keloid disease.

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## Introduction

A keloid is a benign fibroproliferative disorder characterized by abnormal deposition of collagen within a wound. This dermal “tumor” spreads beyond the margin of the original wound, continues to grow with time, commonly recurs following excision, and rarely regress spontaneously.<sup>1</sup> Histology of the keloid specimen reveals collagen bundles arranged in a haphazard manner as compared to the linear arrangement of collagen bundles in the normal skin or wound.<sup>1</sup> There is predominant type 1 collagen with a relatively less type 3 collagen fibers, resulting in a high type 1 to type 3 collagen ratio.<sup>1,2</sup> Keloid is a Greek word meaning crab’s claw. This is due to its clinical appearance that makes it resemble a crab’s claw.

Keloids were first reported in literature in 2000 BC. The report by Smith papyrus described it as firm, nodular, large, and hard swellings on the breast. He described keloids arising both spontaneously and from trauma. He also differentiated keloids from hypertrophic scars. Keloids have been reported to be more common in certain parts of the body, namely, the parasternal areas, earlobes, deltoid areas, and post auricular region.<sup>3</sup> They are less common in the palms of the hand, scrotum, penis, and the upper eyelids.<sup>2,3</sup> The reason for this is not clear, with suggestions that they are prone to occur in areas of high skin tension or repeated trauma. They are more common in Asians and blacks with an incidence ratio of 5:1 and 15:1, respectively, than in the Caucasians.<sup>1–4</sup> Individuals with HLA B14, BW16, DR5, and blood group A are more predisposed to keloid formation.<sup>1,5</sup>

Keloids were thought not to occur in the albinos. However, a recent study conducted in Kenya demonstrated equal incidence of keloids in albinos and nonalbinos populations, suggesting that the presence of melanin may not be critical in the formation of keloids.<sup>6</sup>

The treatment modalities for keloids are many, probably implying that none is perfect. They range from intralesional injection with steroids, five fluorouracil, cryotherapy, and bleomycin injections.<sup>1,4</sup> Surgical management with superficial radiotherapy or steroid injections provides an alternative form of therapy for extensive keloids.<sup>1,3,4</sup> Recurrence rate for keloids ranges from 20% to 100% depending on the various modalities of treatment.<sup>1,3,4</sup> However, factors influencing recurrence of the keloids have not yet been determined. The pathophysiology of keloids is still unclear. The predominant cells implicated in keloid formation are the proliferative and inflammatory cells including fibroblasts, myofibroblasts, macrophages, lymphocytes, and mast cells.<sup>1,7</sup> Understanding the pathophysiology of keloid formation is critical in directing the various pharmacological agents and thus reducing or preventing keloid formation or recurrence.

## Methods

A Medline PubMed search was performed on review articles on the pathophysiology of keloids in English published media. The search words were keloids pathophysiology, fibroblasts, inflammatory cells, cytokines, and genetic factors. Articles published during 1990 to date were analyzed. The relevant conclusions were summarized on the possible causation of keloids, with emphasis being on the role of proliferative, genetic, or inflammatory cells.

## Results

A Medline PubMed search on publications of keloids pathogenesis revealed 335 publications. Of these publications, 68 had original studies relevant to the pathogenesis of keloids. The others were either case studies or studies on keloid management. The study topics and publications identified had a genetic cause of keloids, inflammatory cells as well as fibroblasts and myofibroblasts on keloid etiology, cytokines and humoral factors in keloid formation, and stem cells in the pathogenesis of keloids. All the articles were scrutinized and their findings summarized on their possible role in keloid formation as demonstrated below. The possible etiologies of keloids were analyzed as inflammatory, proliferative, humoral, or genetic in nature. Other possible etiologies included mesenchymal stem cells and melanin.

### *Inflammatory cells*

Keloid formation is partly thought to be due to an exaggerated and prolonged inflammatory phase that may result in sustained release of cytokines and growth factors, which, in turn, stimulate fibroblasts to proliferate and deposit excessive extracellular matrix (ECM).<sup>7,8</sup> Experimental studies have demonstrated that a prolonged inflammatory period involving immune cell infiltrate increases fibroblast activities with greater and more sustained ECM deposition, thereby leading to keloid formation.<sup>7</sup> This finding has been supported by histological findings, which have demonstrated the presence of inflammatory cells in many keloid tissues.<sup>7–9</sup> The inflammatory cells found in high concentration in most keloid specimens are macrophages, neutrophils, mast cells, Langerhans cells, and lymphocytes.<sup>7–10</sup>

### *Macrophages*

Macrophages play a critical role in wound healing. They are classified into two types: M1 and M2.<sup>11</sup> Both are critical in wound healing, with M1 promoting inflammation, while M2 seems to decrease inflammation and promote tissue repair.<sup>12</sup> During the inflammatory phase, macrophages are responsible for the phagocytosis of foreign materials and microorganisms. They also work as antigen-presenting cells (APCs) responsible for the stimulation of the T-cell arm of immunity. They release various cytokines that are critical in tissue repair. M1 macrophages secrete inflammatory cytokines such as IL-12 and TNF- $\alpha$ , whereas M2 macrophages are known to secrete anti-inflammatory cytokines, IL-2, and TGF- $\beta$ , which is strongly implicated in keloid formation.<sup>11</sup> Some common immunohistochemical markers for macrophages include CD68 and CD163.<sup>11,12</sup> Bagabir *et al* demonstrated an increase in macrophages in the keloidal tissues at both intra- and perilesional levels.<sup>9</sup> Shaker *et al* also noted close proximity between the macrophages and the fibroblasts, suggesting paracrine activity of the cytokines released by the macrophages.<sup>13</sup> Dean *et al* demonstrated keloid specimens to have a higher concentration of macrophages and lymphocytes than the normal skin.<sup>14</sup> Other studies conducted by Xuechuan Li and Yu wang *et al* also demonstrated M2 macrophages to be the predominant macrophage cells in keloid specimens.<sup>15</sup> They also found NR3c1, a marker for glucocorticoid receptor, to be closely related to M2 markers in the keloid specimens, suggesting that keloids with predominant M2 markers could be more sensitive to steroid injections.<sup>15</sup> Jin Q *et al*, in the examination of keloid macrophages and normal skin macrophages, found keloid tissue to have significantly elevated CD4<sup>+</sup> macrophages.<sup>16</sup> The transcription and protein expression of INOS, IL-12, IL-10, and TGF- $\beta$  were higher in the keloid macrophages than in normal skin macrophages. Macrophages in keloid tissues also had higher activation status and were more polarized toward the M2 subtypes.<sup>15,16</sup> However, not all keloid specimens have demonstrated a high concentration of these cells. The densities of macrophages also vary from one keloid to another even in the same individual. Interestingly, macrophages seem to be responsible for tissue regeneration in reptiles such as salamanders.<sup>17</sup> Salamanders depleted of macrophages were unable to have their tail regenerate.

### *Lymphocytes*

Lymphocytes are responsible for the cellular arm of the immune response. Lymphocytes are activated by antigen-presenting cells (APCs) after tissue injury, and their role in keloid formation is un-

clear. Immune cells have been shown to be present in keloid specimens, and it is believed that intrinsic fibroblast abnormalities interacting with the immunological response may result in keloids (pmid 10491-047). Martin and Muir demonstrated not only an increase in the T-lymphocytes in the keloids but also a high CD4:CD8 ratio.<sup>18</sup> In addition, aggregation of lymphoid tissue in the keloid referred to as the keloid-associated lymphoid tissues in at least 15% of the histological specimens was also observed.<sup>7,18</sup> Dean *et al* demonstrated keloid specimens to have a higher concentration of lymphocytes than the normal skin.<sup>15</sup> B-cells were, however, not shown to be increased in the keloid specimens. While lymphocytic infiltration in most malignant tissues is associated with good prognosis, the same has not yet been demonstrated with keloid tissue. One may, however, hypothesize that keloid specimens with a high concentration of T-cells are likely to be more aggressive with high possibilities of recurrence due to a strong inflammatory response.

Lymphocytes may further influence wound healing and keloid formation through the release of cytokines. Patients with a keloid have been shown to have high serum concentration of inflammatory cytokines such as IL-6 and IL-17,<sup>19,21</sup> suggesting an indirect role of lymphocytes as the primary movers in keloid pathogenesis.<sup>19–24</sup> IL-6 plays a critical role in both cellular and humoral immune responses. It stimulates the chronic inflammatory response by recruiting monocytes into the wounds.<sup>19</sup> IL-6 is also thought to play a role in the regulation of the stem cell functions through several pathways, leading to the inhibition of apoptosis and maintenance of hematopoietic stem cells.<sup>20</sup> It is thought to play a role in malignant transformation as established during the transformation of colitis to colonic cancer. IL-6 and TGF- $\beta$  are also important cytokines that are involved in Th17 differentiation, and the effects of IL-6 are thought to be amplified by IL-17.<sup>24</sup> Qunzhou *et al* demonstrated IL-6 to be considerably increased in keloids than in the normal skin.<sup>21</sup> They further demonstrated the ability to reproduce keloid-like tissue in immunocompromised rats by using keloid-derived stem cells and IL-6.<sup>21</sup> These activities were abrogated by IL-6-neutralizing antibodies.<sup>21</sup> Lymphocytes may thus influence keloid formation by producing sustained amounts of proinflammatory cytokines in tissues.

Hu Jia *et al* demonstrated keloid specimens to have a high concentration of lymphocytes and fibroblasts in the superficial dermis compared to the deep dermis in histological specimens.<sup>25</sup> These high lymphocyte concentrations were also much higher than those in normal skin specimens, suggesting that the superficial dermis could be the precursor for keloid formation after interactions between the lymphocytes and fibroblasts. In addition, keloid specimens have also been demonstrated to have a high concentration of memory T-cells.<sup>26</sup> The memory T-cell phenotype is known to provide a rapid and highly effective immune response against both bacterial and viral pathogens. The specific memory cells identified were the CD3<sup>+</sup>CD45RO<sup>+</sup>T-cells. CD8 T-cells were also increased. Interestingly, the keloid specimens had a low concentration of regulatory CD4<sup>+</sup>CD25 T-cells, suggesting that keloid pathogenesis could be a result of a local imbalance of Tregs and that correction of this imbalance might represent a therapeutic approach to keloid fibrosis (pmid 24617809). In contrast, other studies have demonstrated that the M2 macrophage in keloid tissues can promote Treg differentiation by upregulating the FOXP3 expression (pmid 29253537). Additional studies are therefore needed to understand the role of Tregs in keloid tissues and whether the frequency of these cells may vary between keloid tissues and in the peripheral blood of patients.

### Mast cells

Mast cells (MCs) are an important part of the innate immune system and are abundant in the skin. There is growing evidence that MCs play a critical role in wound healing and possibly keloid formation.<sup>27–30</sup> MCs have been found to be increased in most keloid tissues as compared to those in the normal skin or scar tissues.<sup>1,7,10</sup> MCs are thought to be responsible for the symptoms of itchiness in the keloids. In wound healing mouse models, scarless wound healing was noted in mice fetus with no MCs at the embryonic age of 15 days as compared to scar healing in mice at the embryonic age of 18 days with MCs.<sup>31</sup> Shaker *et al.* also demonstrated MCs in close contact with fibroblasts, suggesting a paracrine activity.<sup>13</sup> *In vitro* studies with MCs and fibroblasts have demonstrated that MCs increase fibroblast proliferation and collagen synthesis.<sup>32</sup> MCs have also been thought to produce cytokines and other factors such as histamine that can also stimulate fibroblast activities and hence collagen deposition and scar formation.<sup>27,28</sup> Further studies have also shown that there is a correlation between absolute MC count in the wounds with scarring and fibrosis.<sup>27–29</sup> A large number of MCs

have also been demonstrated under other inflammatory conditions such as Crohn's disease and liver cirrhosis.<sup>33</sup> Incidentally, MCs have been shown to be inversely proportional to the tumor size and mitotic activities in patients with dermatofibrosarcoma.<sup>34</sup>

The MC products such as histamine and heparin may play an important role in initiating a series of biochemical events, leading to hypertrophic scarring and keloid development. Elevated histamine levels may enhance the rate of collagen synthesis, while high heparin levels increase the degree of vascularization in a developing keloid. The incidence of keloids depending on race, gender, and age can be positively correlated with serum IgE levels.<sup>30</sup> This may implicate MC hypersensitivity in keloid development.

The hypothesis that IgE-mediated MC hypersensitivity may be an important factor in the development of keloids and hypertrophic scars was tested by surveying 1206 adolescents for the coincidence of keloids or hypertrophic scars with a wide variety of allergic symptoms.<sup>30</sup> A statistically significant increase in the incidence of allergic symptoms was found for both keloid formers and hypertrophic scar formers as compared to that in normal respondents. Keloid formers showed the highest incidence of allergic symptoms. Therefore, the IgE-mediated release of the MC products histamine and heparin may play a role in the development of keloids and hypertrophic scars. Lu *et al.* in a retrospective study in patients with atopic dermatitis in Taiwan also found these patients to have a threefold incidence in keloid formation as compared to the normal population, strongly suggesting an immune reaction in the pathophysiology with the MCs<sup>30</sup>.

### Fibroblasts

As the primary stromal cells in the body, fibroblasts are/ or rather make the “glue” that holds everything together. These cells are ubiquitous and contribute to the structure of nearly every tissue in the body, both as cellular constituents and as synthesizers of ECM (pmid 30468625). Fibroblasts are considered the main proliferative cells in the body and are responsible for the deposition of collagen fibers and ECM responsible for wound healing. Fibroblasts are also thought to be the main inductive cells for keloid formation.<sup>7</sup> However, the exact mechanism by which fibroblasts potentiate keloid scar formation and invasion is unclear. Fibroblasts are mobilized to wounds by growth factors and cytokines from the platelets, macrophages, and lymphocytes in the early phases of wound healing.<sup>1,2,7</sup> These growth factors may also stimulate resident stem cells to differentiate into fibroblasts.<sup>35,36</sup> Once recruited, fibroblasts are stimulated to synthesize ECM and collagen fibers.

Evidence suggesting fibroblast as the predominant cell in keloid synthesis includes the fact that keloid tissues have been shown to have a high concentration of fibroblasts than normal tissues.<sup>2,7</sup> Although they seem to have the same rate of apoptosis as that of the normal fibroblasts, their proliferation rate is higher, resulting in a high density of fibroblasts in the keloid tissues.<sup>37</sup> Further evidence alluding to fibroblast as the primary mover in keloid pathogenesis was illustrated by Ashcroft *et al.* who demonstrated apparent apocrine activity of the fibroblast by extracting culture media of the keloid fibroblast and cultured normal fibroblasts, scar fibroblasts, perilesional fibroblasts, and intralesional fibroblasts.<sup>38</sup> All the fibroblasts in the cultured media demonstrated rapid proliferation and decreased apoptotic rates, suggesting that keloid fibroblasts could release cytokines that recruit the surrounding fibroblasts to proliferate and increase collagen synthesis.

Keloids fibroblasts have been shown to be more sensitive to growth factors, especially TGF- $\beta$ 1 and  $\beta$ 2 and platelet-derived growth factor (PDGF) than the normal fibroblasts.<sup>38</sup> TGF- $\beta$ 1 and  $\beta$ 2 are thought to be the main cytokines responsible for wound healing by fibrosis.<sup>17,39,40</sup> They have been incriminated in not only keloid formation but also other fibrotic conditions such as pulmonary fibrosis, schistosomiasis, and cystic fibrosis.<sup>41</sup> TGF- $\beta$  works through the SMAD pathway leading to the transcription of mRNA, resulting in protein synthesis and collagen formation. The effect of TGF- $\beta$ 1 and  $\beta$ 2 on keloid fibroblasts has been demonstrated *in vitro*<sup>42,43</sup> and shown to have a predominant stimulatory effect on the keloid fibroblast than on the normal tissue fibroblasts, with keloid fibroblasts producing large amounts of collagen compared to the normal fibroblasts.<sup>16,42,43</sup> This effect can be blocked by the use of anti-TGF- $\beta$  antibodies. The high sensitivity and increased production of collagen by the TGF- $\beta$ 1 and  $\beta$ 2 ON the keloid fibroblast is thought to be due to the higher expression of receptors on the keloid fibroblasts.<sup>44</sup>

Effects of fibroblasts seem to be influenced further by tenascin C.<sup>45</sup> Tenascin C is a glycoprotein encoded by the TNC gene. Tenascin C is upregulated during inflammatory conditions, tumorigenesis, and embryogenesis. Tenascin C acts as a chemokinetic agent that promotes fibroblast survival, distribution, and antiapoptotic effects.<sup>45,46</sup> Keloid specimens have been shown to have higher and persistent concentrations of tenascin C.<sup>45</sup> This seems to promote collagen synthesis and fibrosis. Another protein that seems to influence the effects of the fibroblasts is decorin.<sup>7,46</sup> Decorin is a protein encoded by the DCN gene. It has been shown to downregulate the effects of growth factors such as PDGF and thus inhibit angiogenesis and probably hypertrophic scars and keloid formation.<sup>47</sup> This has been demonstrated by the findings that keloid and hypertrophic scar fibroblast specimens have been shown to have low concentration of decorin as compared to those in the normal tissues.<sup>7,48</sup>

### Myofibroblasts

Myofibroblasts were identified initially in the granulation tissue of healing wounds.<sup>49</sup> They are contractile cells expressing many of the morphological and structural features of smooth muscle cells, with flattened and irregular morphology and well-developed cell–ECM interactions and intercellular gap junctions. In particular, they have abundant expression of  $\alpha$ -SMA.<sup>49–51</sup> The classical description of the differentiation of the myofibroblast from resident fibroblasts involves their passing through a proto-myofibroblastic stage.<sup>51</sup>

Immunohistochemically, they are demonstrated by stains for the alpha-smooth muscle actin.<sup>7,51</sup> Myofibroblasts have been demonstrated in not only wounds but also many tissues such as the liver, lungs, and kidneys and are thought to be responsible for healing by fibrosis.<sup>50,51</sup> In wound healing, myofibroblasts assist in contracting the wound through intracellular contraction and thus pulling the wound edges together.<sup>48,49</sup> They also assist in the production of ECM including collagen type 1 and 3 fibers. They are thought to be the main cells responsible for tissue healing by fibrosis.<sup>51</sup> Myofibroblasts are thought to originate from either the pericytes, fibroblasts, smooth muscle cells, or the fibrocytes.<sup>49–51</sup>

Myofibroblasts have been demonstrated by some studies to be more common in keloids than in other tissues including hypertrophic scars, suggesting that they could play a role in keloid formation. They have been reported in approximately 33% of the keloid specimen.<sup>7,52</sup> In hypertrophic scars, on the other hand, their density has been shown to regress once the wound fully heals, with some authors reporting no myofibroblasts in hypertrophic scars.<sup>52</sup> Myofibroblast differentiation is thought to be due to the effects of TGF- $\beta$ 1, which stimulates fibroblasts to synthesize  $\alpha$ -SMA, resulting in myofibroblast formation.<sup>52</sup> IL-6 has also been shown to have great influence on the transformation of the fibroblasts into myofibroblasts, strongly suggesting the influence of inflammatory cells on myofibroblasts' activities.<sup>53</sup> Myofibroblast development is also thought to be profoundly influenced by the mechanical microenvironment, in particular, by the organization and stiffness of the ECM.<sup>54</sup> These observations, probably explains why keloid and hypertrophic scars are common in areas of skin tension such as the chest and the arms.

Myofibroblasts have been implicated in two theories of keloid formation: the stress/tension theory and the hypoxia theory. In the tension theory, fibroblasts, when put under stress or tension as occurs in wounds, are thought to transform into myofibroblasts under the influence of TGF- $\beta$ 1. This finding has been demonstrated *in vitro* when fibroblasts differentiated into myofibroblasts by provision of TGF- $\beta$ 1, extracellular stress, and provision of special ECM.<sup>54</sup> In the hypoxia theory, hypoxia is thought to stimulate the transformation of fibroblasts into myofibroblasts, which are then responsible for healing by fibrosis.<sup>27</sup> Ammendola *et al* demonstrated in *in vitro* studies that hypoxia drives the transition of human dermal fibroblasts to cells with a myofibroblast-like phenotype through the TGF- $\beta$ 1/smad 3 pathway.<sup>27</sup> Keloid tissue was also demonstrated by the same authors to be significantly hypoxic compared to the normal tissues by high expression of the hypoxia-inducible factor alpha.<sup>27</sup> Myofibroblast may thus be a critical cell in the formation of keloids. In histological specimens, keloid blood vessels were noted to be narrower and partially obliterated by the myofibroblasts and the endothelial cells, probably resulting in a hypoxic environment that is thought to be responsible for keloid formation.<sup>50</sup> Many tissues and pathologies have been described in which myofibroblasts have been identified, including hypertrophic and keloid scars in the skin, fibrotic liver as seen in liver cirrhosis and other liver pathologies, renal fibrosis, and idiopathic pulmonary fibrosis.<sup>4</sup> More recently, cells with pheno-

typic features of myofibroblasts have also been found in and around a number of epithelial tumors, where they have been termed cancer-associated fibroblasts or stromal myofibroblasts.<sup>5–7</sup>

During skin wound healing, fibroblasts in the vicinity of the injury become activated, resulting in a phenotype that is proliferative and motile; these cells migrate to the site of injury, convert to myofibroblasts, and synthesize a robust ECM to aid in wound repair.<sup>32</sup> Once the tissue has been repaired, these myofibroblasts undergo apoptosis, or in some instances, adopt a quiescent state. However, it remains unclear how these processes are controlled and regulated.<sup>51</sup> In some situations, it is thought that the active myofibroblasts at the site of injury may lead to abnormal scarring including hypertrophic scars or dermal keloids (sarraza *et al* 2011).

#### *Keloids and Acne inflammation*

Acne is a chronic inflammatory disease associated with scar development in some patients.<sup>55</sup> However, not everyone with acne develops scars. Currently, it is not possible to predict who will develop a scar or even which acne lesion is most likely to develop as a scar. Several factors including genetic factors likely predetermine the inflammatory response and, ultimately, the wound healing process of acne lesions.<sup>56</sup> The type of inflammatory response can be different between scar-formers vs. non-formers, and the process may be due to an altered wound healing process similar to what is seen in patients with a keloid.<sup>57</sup> The presence of persistent inflammation leads to granulation, tissue repair, angiogenesis, and scar formation; if this process is relatively slow, then continuous hyperplasia will result in hypertrophic scar.<sup>58</sup> Some studies have observed patients presenting keloids due to previous acne characterized by irregular nodules, hard plaques, and bright red lesions on the face.<sup>58,59</sup> From this point of view, considering the persistence of inflammatory factors in keloids, this inflammatory response could be alleviated by reducing sebaceous gland secretion by oral isotretinoin. For example, a patient with keloids on his chest for 20 years that had become painful during the previous six months mentioned that he could eject sebum-like material from the reactive keloid. Extensive sebaceous gland hyperplasia was evident on his face, suggesting a link between acne and keloids.<sup>58</sup> However, scholars now believe that this condition may reflect only hyperplasia of sebaceous glands. Therefore, the use of oral isotretinoin to inhibit sebaceous gland secretions is a reasonable therapeutic strategy for acne, and whether this may have an effect against keloids remains an area of active research.<sup>60–61</sup>

#### *Genetic composition and keloid disease*

The occurrence of keloids has an equal sex distribution. The highest incidence of keloids is in the second to the third decade.<sup>1,5,6</sup> Keloid formation is seen in individuals of all races including albinos, but dark-skinned individuals have been found to be more susceptible to keloid formation, with an incidence of 6% to 16% in African populations.<sup>1,2,4,–6</sup>

The concept of genetic predisposition to keloids has long been suggested because patients with keloids often report a positive family history.<sup>62</sup>

Keloids with a familial tendency tend to present early and occurs at multiple sites. Bayat and colleagues compared the profiles of patients of Afro-Caribbean origin with keloid scars at single versus multiple anatomical sites and found the latter to be more common in younger age groups and in females.<sup>63</sup> Another important finding was that more than 50% of all patients with keloids had a positive family history of keloid scarring. Family history was also strongly associated with the formation of keloid scars in multiple sites as compared to those at a single anatomical site.

Marneros and colleagues studied two families with an autosomal-dominant inheritance pattern of keloids and identified linkage to chromosome 7p11 and chromosome 2q23 for the African and Japanese families, respectively.<sup>64</sup>

Brown and colleagues found a genetic association between HLA-DRB1\*15 status and the risk of developing keloids in white individuals.<sup>65</sup> Moreover, carriers of HLA-DQA1\*0104, DQB1\*0501, and DQB1\*0503 have been reported to be have an increased risk of developing keloid scarring.<sup>65</sup>

The proposed pathway to keloid formation includes protein kinase, transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), interleukin 6, and plasmin-activating inhibitor.<sup>34,66</sup> However, Bayat *et al* in the analysis of the serum concentration of TGF- $\beta$ 1 and - $\beta$ 2 among patients with keloids, hypertrophic scars, and normal skin found out that there were no significant changes in their serum. He further demonstrated

that the TGF- $\beta$ 1 gene alleles in keloid formers and normal controls were the same, putting to doubt abnormalities in TGF- $\beta$ 1 genes as a precursor to keloid formation.<sup>67</sup>

#### *Melanocytes and keloid formation*

Melanocytes have all along been thought to have a role in keloid formation. Pointers to this include the fact that keloids are more common in blacks and other people with dark skin who have a higher concentration of melanin. They also seem to be rare on the sole and palm even in populations where they are endemic. The mechanism on how melanocytes stimulate keloid formation is still not clear, given the fact that keloids tend to form in the dermal layer as compared to the epidermal layer where melanocytes are abundant. An experimental study involving fibroblasts and melanocytes showed an increased fibroblast activity with increased collagen synthesis as compared to the controls.<sup>68</sup> Interestingly, an epidemiological survey on keloids in a population in Kenya demonstrated the incidence of keloids to be same as that among the normal population and the albinos, strongly suggesting that melanin may not have much role in keloid formation.<sup>6</sup>

### **Discussion**

The etiology and pathogenesis of keloid disease is still unknown. Many theories have been proposed including genetic, proliferative, inflammatory, hypoxic, and mechanical tension stress theories.<sup>1,2,3,7</sup> None of these theories have, however, been proven. Because of the lack of proper understanding of the disease, there are no standardized management protocols, with different centers managing the disease differently. The recurrence rates are high as a result of this, with some centers reporting a recurrence of up to 50%.<sup>1,2,5</sup>

Although genetics seems to play a significant role in keloid formation as evidenced by a good proportion of patients with familial disease, an equal number of patients have spontaneous keloids with no known family history of the disease, suggesting a multifactorial etiology. Other factors pointing to the genetic predisposition include the fact that patients with blood group A seem to be more prone to keloid formation, suggesting a possible role of antigen A in the red blood cells.<sup>1,7</sup>

The disease is more common in blacks, followed by the Asians and, finally, in the Caucasians, pointing to the fact that the severity of the melanin pigment may play a role in the pathogenesis.<sup>1,2,66</sup> Further evidence suggesting the role of melanin in keloid formation includes the fact that keloids rarely form on the sole or palm areas, which have less melanin density. They tend to be common in adolescents and pregnant women, probably due to the influence of melanocyte-stimulating hormone that stimulates melanin production and deposition.<sup>66</sup> Proponents of the melanocyte theory hypothesize that, during injury, the melanocytes migrate from the basal layer and interact with the fibroblasts, stimulating them to proliferate and produce large amounts of collagen fibers. This theory has been supported by experimental work where fibroblasts were cultured with melanocytes with a control of fibroblasts alone and their collagen synthesis analyzed. Fibroblasts cultured with melanocytes had large deposits of collagen compared with those in the control.<sup>66</sup> Earlier studies had also indicated that keloids were rare in albinos. However, a cross-sectional study by Kiprono et al demonstrated that the incidence of keloids in albinos and normal population was actually the same, negating the melanin theory.<sup>6</sup>

Fibroblasts have been considered the main cells in keloid formation, probably because they are the main cells responsible for ECM synthesis in the body. (2,7) Keloid fibroblasts are thought to be genetically programmed to synthesize large volumes of collagen fibers, a hallmark of keloid disease.<sup>2,7,37,38</sup> Factors suggesting fibroblasts to be the main cells in keloid formation include the fact that keloids seem to have a higher concentration of fibroblasts than the normal tissues. Keloid fibroblasts seem to proliferate more than normal fibroblasts. Further, they seem to respond more to growth factors than those in normal tissues, such as TGF- $\beta$ 1.<sup>37,38</sup> Keloid fibroblasts also seem to have an apocrine activity that seems to stimulate or recruit nearby fibroblasts for collagen synthesis.<sup>39</sup> However, studies have also shown that normal fibroblasts subjected to continuous stimulation by cytokines such as TGF- $\beta$ 1 would lead to overproduction of collagen fibers akin to keloid fibroblasts.<sup>40,41</sup> One could therefore hypothesize that collagen overproduction by the keloid fibroblasts is not necessarily due to genetic



predisposition but as a result of continuous stimulation by the cytokines and growth factors, resulting in overexpression of genes responsible for collagen synthesis and abundance of collagen.

Myofibroblasts are closely related to the fibroblasts. The source of myofibroblasts in keloid disease is still unclear.<sup>51</sup> They are thought to be responsible for the contraction of the wounds and tend to regress once the scar tissue matures. They are, however, persistent in the majority of keloid specimens. Myofibroblasts have been shown to be responsible for contraction of wounds. The origin of myofibroblasts in keloids and wounds in general has not been clear, with some authors suggesting that they originate from stem cells in wounds, while others suggesting that they form from fibroblasts when under either mechanical stress or hypoxia. (51,52.) (This has been shown experimentally by putting fibroblasts under mechanical stress.) Keloids have also been shown to have a higher concentration of hypoxia-inducible factor alpha,<sup>27</sup> giving credit to the hypoxia theory. Myofibroblasts have, however, been identified not only in keloids but also in any tissue that heals by fibrosis, including the kidneys in glomerulonephritis and liver cirrhosis, suggesting that their presence in keloids may not necessarily be attributed to the formation of the same.<sup>7</sup>

Inflammation, as the prime mover of keloid formation, seems to have taken a back seat in most theories on keloid formation, with emphasis being on the proliferative phase. However, there is now increasing evidence suggesting that inflammation could play an important role in keloid formation if not could actually be the initiator of keloid formation. (7–12.) Histological studies on keloid specimens have shown most of them to have high concentration of inflammatory cells, especially macrophages, lymphocytes, and mast cells, suggesting a possible role in the formation of the same.<sup>7</sup> Further normal fibroblasts in culture media with macrophages have shown them to produce large volumes of collagen similar to keloid fibroblasts, strongly suggesting the critical role they play in keloid pathogenesis.<sup>13</sup> Mast cells in *in vitro* studies with fibroblasts have been shown to increase fibroblast proliferation and collagen synthesis.<sup>28,29</sup> Mast cells have also been shown to be in high concentration during other fibrosis conditions of the body.<sup>28</sup> Keloid specimens have also been shown to have high concentration of memory T cells, with lower concentration of the regulatory cells. Anecdotal studies have also shown more patients with keloids to also have allergies, suggesting an auto immune disorder.<sup>30</sup> Patients with keloids have also been shown to have a high serum concentration of pro-inflammatory cytokines such as interleukin-6 and TGF- $\beta$ 1, which, in experimental works, have been used to produce keloid-like fibroblasts (19,20,), suggesting their critical role in keloid formation. Other pro-inflammatory markers in keloid tissues include tenascin C, which has been found in high concentration in keloid specimens than in normal tissue. Tenascin C has been shown to potentiate the action of TGF- $\beta$ 1 on the fibroblasts, promoting them to synthesize collagen fibers.<sup>45,46</sup> One may therefore hypothesize that patients who form keloids have a strong inflammatory response in any form of injury. The prolonged and strong response results in the production of inflammatory cytokines such as interleukin-6 and TGF- $\beta$ 1 and - $\beta$ 2, which, in turn, leads to the activation of fibroblasts, leading to the overproduction of ECM.

## Conclusion

There is still no consensus as on what are the main driving cells to keloid formation. There is high possibility that keloid formation is multifactorial in nature, with the inflammatory cells, macrophages, lymphocytes, and mast cells playing a critical role than previously thought. In predisposed individuals, these cells seem to produce large quantities of cytokines such as interleukin-6, -17, and TGF- $\beta$ 1 and - $\beta$ 2, which, in turn, stimulate fibroblasts to synthesize large volumes of collagen. The oversensitized fibroblasts, in turn, recruit the surrounding fibroblasts through paracrine release of other cytokines, resulting in a vicious cycle of collagen synthesis and deposition, a characteristic of keloid disease.

## Declaration of Competing Interest

The authors state no conflict of interest.

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