IMMUNOLOGIC ASSOCIATIONS OF KEOIDS


Wound repair results in a spectrum of lesions ranging from nearly "scarless" healing to pathologic scars. Keloids are the sequelae of abnormal healing processes. Keloids consist of scar tissue that extends beyond the confines of the original wound (1). Keloids tend to form within the first year after the inciting skin wound. In many situations, the history fails to identify any preceding injury. The presence of foreign material, hematoma, infection, inflammatory changes (2) or increased skin tension (3) are associated with a susceptibility to keloid formation. Keloids have a marked tendency to recur after excision. Keloids may become pruritic, tender and painful. The symptoms and clinical behavior of keloids are suggestive of an ongoing inflammatory process.

Despite extensive research, the epidemiologic, histologic, endocrinologic and biochemical findings are not readily applicable to the diagnosis or management of keloids at the present time. The cause of keloids remains an enigma; however, there is evidence to support a role for immune mechanisms in the pathogenesis of keloids. An understanding of the inflammatory changes contributing to keloid formation may be essential to understanding scarless wound healing.

EPIDEMIOLOGIC FACTORS

Humans are the only creatures affected by keloids (4). Patients between the ages of ten and 20 years demonstrate the highest incidence of keloid development (5). They occur in all age groups but are rarely found in newborns (6). The prevalence in the general population is unknown. Darkly pigmented individuals are affected more often than those with fair skin. Europeans residing in the tropics acquire an increased likelihood of having keloids (7). The reported ratios of black to Asian to white as well as male to female vary considerably (5, 8, 9). A family history of keloids is frequently elicited (10) and autosomal recessive (11), autosomal dominant (12) and cross-linked recessive (13) patterns of inheritance have been proposed.

HISTOLOGIC FACTORS

Keloids are likely to occur on the upper one-half of the body—the head, neck, chest, shoulders and arms (14). The most commonly affected areas are the preternal, lateral cheek in females and skin overlying the mandible, neck and ear. Skin with modified dermal layers (eyelids, areola, penis and scrotum) (15), in addition to skins lacking sebaceous glands (16) and melanocytes (palms and soles) (17), are rarely affected by keloids. Melanocytes are absent or present in only trace amounts in fibrotic lesions (including keloid) (18). There are no known reports of keloids in albinos.

There are no standardized criteria in establishing the histopathologic diagnosis of keloids. Histologic definitions are vague and subjective (5, 19–21). A few communications suggest a continuum of disease progressing from hypertrophic scar to keloid (1, 2, 20). Several authors now believe that they can use the electron microscope to distinguish keloids from hypertrophic scars (22–24).

ENDOCRINOLOGIC FACTORS

Endocrine factors have been implicated in the cause of keloids (25, 26). The ages of the greatest incidence of keloid correlate with the periods of physical (puberty) (27) and pituitary growth (28, 29). The predisposition for keloid formation has been associated with abnormal function of the hypothalamus and pituitary (17, 29, 30), thyroid (28, 29) and parathyroid (31). To date, no
one has verified a pathogenetic mechanism demonstrating an endocrinologic cause of keloids; the preceding hypotheses are conjecture based primarily on clinical observations.

Keloids frequently grow rapidly during pregnancy (27) and may regress with menopause (5, 28, 32, 33). A causal relationship of keloid formation and estrogen concentration has not been documented despite multiple investigations (2, 34). Reports of elevated testosterone binding by keloid fibroblasts have been published; the increased androgen metabolism is believed to contribute to keloid pathogenesis (35).

**BIOCHEMISTRY**

Keloid tissues contain increased quantities of water (20, 36, 37), calcium (38), histamine (20, 39, 40), acid phosphatase (41), alanine transaminase, lactate dehydrogenase (42, 43), alpha-globulins (44), fibronectin (45) elastin and glycosaminoglycans (37) and proteoglycans such as chondroitin four sulfate (46). An additional finding that has been repeatedly confirmed is that excessive collagen deposition is a hallmark of keloids (20, 47–50). Keloid derived fibroblasts, in vitro, produce increased amounts of collagen per cell in comparison with normal skin and scar fibroblast strains (51–53). The keloid fibroblasts synthesize excessive amounts of collagen throughout the in vitro lifespan (when replated beyond four passages). It is believed that the fibroblasts are functioning autonomously because repeated subculturing techniques isolate the fibroblasts from the influence of any in vivo factors, that is, in the absence of any humoral substances.

The explanation for the exuberant production of collagen observed in keloids is unknown. The concentrations of galactosylhydroxylysyl glucosyltransferase (50), proline hydroxylase (20, 39, 47, 48, 50), soluble collagen (20, 36) and abnormal collagen cross linkages (54) are also increased in keloid tissue. Furthermore, increased (20), decreased (55) and unchanged (49, 56) amounts of collagenase have been cited in keloidal versus normal skin tissue collagenase assays. The degradation of newly synthesized procollagen polypeptides is decreased in fibroblast cultures derived from keloid tissue when compared with normal skin (37).

Reported changes in the collagen content of keloids include abundant type I collagen (57) versus unaltered quantities of type I collagen (50, 58), excessive type III collagen (57, 58) versus normal amounts of type III collagen (57, 58) and an increased (37, 57) versus an unchanged (50) ratio of collagen type I to type III. Investigators have attempted to corroborate the aforementioned results with findings of increased quantities of fibronectin messenger ribonucleic acid (mRNA) (45); increased (37, 57) versus normal amounts of type I procollagen specific mRNA (50) and normal values of type III and V procollagen specific mRNA (50, 57) and type IV procollagen specific mRNA (50) levels in keloid specimens.

One author noted that there was no difference in total (type I, II and V) procollagen specific mRNA between keloid and control fibroblasts of all patients examined. However, the cultured fibroblasts of keloid tissue exhibited an accelerated rate of type I and III procollagen specific mRNA when compared with the fibroblasts from the normal appearing skin of the same patient (50). The accelerated rate of collagen synthesis by keloid fibroblasts returns to normal if the cells are harvested from lesions that are greater than two to three years of age (60).

Several hypotheses based on the biochemical findings have been advanced to account for the accumulation of collagen by interference with collagen breakdown or by enhancement of collagen production. As mentioned previously, there is an abundance of alpha-globulins (alpha-one-antitrypsin and alpha-two-macroglobulin) in keloid tissue when compared with normal skin or scar fibroblast strains (44). Alpha-globulins inhibit collagenase and may result in decreased collagen degradation. However, the collagenase content of keloids is variable and alpha-globulins do not exclusively accumulate in keloids; they are also found in hypertrophic scars. Individuals have had atypical keloids after dermabrasion while on or after receiving isoretinoin (61), which is known to reduce the production of collagenase (62).

Results of other studies have shown that the deposition of collagen, measured in terms of the
formation of granulation tissue in implanted Ivalon™ sponges, is enhanced by injections of a histamine liberator (63, 64). Keloid tissues contain increased quantities of histamine as previously mentioned (20, 39, 40). Fibroblasts, in tissue culture, produced increased amounts of collagen per cell when incubated with an extrinsic stimulus such as Dilantin® (65). Dilantin has triggered autonomous fibroblast function; Dilantin induced gingival hyperplasia explants demonstrate accelerated rates of collagen synthesis through repeated subcultures in the absence of Dilantin (66). It is as if the trigger selects out or initiates and promotes a hypermetabolic fibroblast subtype or clone. Perhaps histamine or a yet unidentified trigger act in a similar manner.

**IMMUNOLOGIC FACTORS**

There is sufficient evidence to support an immunologic component in the pathogenesis of keloids. Rats that were immunostimulated exhibit enhanced collagen synthesis (67). The slow primary appearance and rapid secondary growth after excision of a keloid have been likened to an immunologic response (exposure and sensitization to an antigen, establishment of memory, reinoculation with the antigen and activation of humoral and cellular immunity) (68). Human leukocyte antigens (HLA) B14, BW16 (69) and blood group A (33) have been affiliated with a predisposition to keloid formation; other investigators have reported no correlation of histocompatibility antigens to the keloid state (70).

*Immunoglobulin, complement and lymphocyte.* Immunoglobulin (Ig) G (70, 71) and IgA and IgM (71) seem to be more heavily deposited in keloid tissue. The reports evaluating serum immunoglobulins and complement levels have been conflicting (Table I). Findings of increased quantities of serum IgG (68) and IgM plus C3 (72) as opposed to data demonstrating normal levels of serum IgG (70, 72) and IgM plus C3, C1Q and C4 (70) as well as assays measuring decreased amounts of serum IgA plus C4 (68, 72) and C3 (68) have been reported to be present in patients afflicted with keloids. The response of peripheral blood lymphocytes to mitogens in patients with keloids have been quantified (72). The extent of the keloid reaction has been correlated with the lymphocytic infiltration of the wound site (73, 74) as well as a T-cell lymphocytosis of the peripheral blood. Investigators have documented a heightened reactivity to delayed hypersensitivity skin tests against old tuberculin and dinitrochlorobenzene; these authors as well as others have concluded that keloid is the sequelae of a hypersensitive cell mediated immune response (75, 76).

*Immunoglobulin E and mast cells.* IgE mediated release of mast cell products may contribute to the development of keloids. The incidence of keloids for race, gender and age, directly correlates with serum IgE levels. In a recent report (77), keloid afflicted subjects had the highest frequency of allergic symptoms when compared with individuals with hypertrophic scars. Histologic findings reveal mast cells interspersed among dermal collagen bundles in keloid specimens (28, 78). Mast cells can be stimulated by IgE to expel cytoplasmic granules containing histamine, heparin, serotonin, acid hydrolase, a neutral protease, chymase and so on (70, 79). Histamine levels are increased in keloid tissue (20, 39, 40) and histamine is capable of enhancing the formation of collagen by in vivo fibroblasts (62). Histamine is also a competitive inhibitor of lysyl oxidase, which could explain the diminished concentration of lysyl oxidase and copper observed in keloid (80). Depressed enzyme (lysyl oxidase) activity may result in abnormal collagen cross linkages (81) and contribute to the increased amount of soluble collagen in keloids (20, 36).

*Autoimmunity.* Experimental data also point to an autoimmune cause. The natural history of keloids is to grow progressively larger with time; keloids that had been transplanted to the nude mouse, diminished in size despite retention of
the original histotype (82, 83). These results suggest that the accelerated growth of autogenous (versus transplanted) keloids may require immunologic stimulation. Another team of researchers stated that they were capable of producing keloids after immunization with autologous skin (84). An autoimmune response to trapped sebum has been assumed (85). Based on this hypothesis, the investigators have claimed to have prepared a vaccine and to have successfully desensitized ten of 11 patients to keloid recurrence (86).

Autoimmune (noncomplement fixing) antifibroblast antibodies (AFA) have been extracted from the lymphocyte isolates of every keloidal patient examined in a recent study; these antibodies were specifically directed against the fibroblasts of the individuals and were not detected in the lymphocyte eluates of subjects with normal or hypertrophic scars (87). The authors hypothesized that the AFA have a fibroblast stimulating role in the pathogenesis of keloid. One of the same investigators described the presence of similar antibodies in patients with psoriasis; these antibodies are directed against psoriasis specific nonhistone proteins. This discovery suggests a possible mechanism by which antibodies may exert control over the expression of the gene responsible for psoriasis (88, 89). Noncomplement binding AFA have also been reported to occur in an instance of hematoporphyrin-induced keloid (90). Other investigators did not detect the presence of AFA in five patients with keloids (91).

Immunologic mediators. The influence of immunologic mediators (cytokines and peptide growth factors) must also be considered. Excellent reviews detailing the role of these substances in wound healing are available (92, 93). These substances (lymphokines, monokines, growth factors) affect fibroblast proliferation, chemotaxis and collagen, collagenase and hyaluronic acid production (94). Recent reports have focused on the alterations of cytokine production in patients with keloids. Peripheral blood mononuclear cell fractions from black patients with keloids demonstrate depressed production of interferon-alpha, interferon-gamma and interleukin-2 and enhanced production of tumor necrosis factor alpha and interferon-beta when compared with normal patients (95).

Some of these agents promote collagen accumulation. Interleukin-1, a monokine, stimulates collagen production (94). Antigen stimulated T-cells produce lymphokines that are capable of stimulating fibroblast proliferation and collagen synthesis (96). Accumulations of T-cells in close association with fibroblasts have been described in the keloid-like lesions of patients with progeria. It is believed that these activated T-cells interact with fibroblasts through a lymphokine intermediary, resulting in accelerated fibroblast growth and production of unusual amounts of type IV collagen (74, 97). Enhanced collagen deposition by cultured fibroblasts has been reported when lymphokine-rich supernatants, derived from phytohemagglutinin (PHA) stimulated cells, were added to the fibroblast medium (98). These lymphokines have been identified and characterized as a 100,000 to 170,000 molecular weight lymphokine (99) and transforming growth factor beta (100, 101). Transforming growth factor beta has also enhanced the activity of epidermal growth factor on keloid derived fibroblast cultures (102). Adult rat wounds treated with neutralizing antibody to transforming growth factor-beta have reportedly healed without formation of scar tissue (103).

Substances derived from leukocytes can also limit the deposition of collagen. Lymphocytes can produce collagenase (104). Monokines can decrease collagen deposition—interleukin-1 and tumor necrosis factor alpha stimulates fibroblast collagenase production (93, 105), while beta interferon inhibits collagen production (106). A human monocyte-macrophage factor, labeled mononuclear cell factor (MCF), which is capable of stimulating collagenase synthesis by cultured fibroblasts, has been described (107, 108). T-cell lymphocytes or immunoglobulin interact with monocytes-macrophages to modulate the production of MCF. Human MCF is similar to murine interleukin-1. Stimulated T-cell lymphocytes produce lymphokines such as alpha and gamma interferon that can inhibit fibroblast collagen production (99, 106, 109).

Results of preliminary studies have shown that immunotherapy may have a role in the treatment of keloids. Investigators have injected gamma interferon into keloidal and hypertrophic lesions; they claim that the treatments produced at least a 50 percent reduction in the size of five of ten scars studied, with no serious toxic side effects (110, 111). Another group of investigators have confirmed these results, using intralesional recombinant interferon-gamma in the treatment of keloidal scarring (112). Intralesional injections of interferon-alpha-2b have produced reduction in the size of a progressively enlarging keloid
Topical cyclosporin resulted in uniform reductions in T-cell numbers in keloidal scars (114). The use of neutralizing antibody to transforming growth factor-beta was previously mentioned (103).

**FIBROBLAST HETEROGENEITY**

Intrinsic fibroblast abnormalities interacting with the immunologic response may result in keloids. Results of experimental studies have shown that keloid fibroblast strains demonstrate an autonomous capacity to synthesize large quantities of collagen (52). These fibroblast cell lines were subcultured more than three passages and, therefore, were performed in the absence of any in vivo humoral factors.

Heterogeneous fibroblast populations are likely to be present within wounds at the same time (synchronous). Studies on cloning have revealed heterogeneity with respect to the growth potential of human fibroblasts (115). Metabolic differences among human fibroblasts have been identified; these cells, when cultured and replated, will maintain their differentiation through late passage (116). Cultured fibroblasts harvested from the dermis of patients with progressive systemic sclerosis produce more collagen than normal cells (117). Subpopulations of fibroblasts may also vary with the age of the wound (metachronous). For example, myofibroblasts are abundant in the early stages of wound healing (third week), but rapidly diminish in number by the 20th week (118). Myofibroblasts are not abundant in mature keloids (118).

The different subpopulations are likely to express different antigenic surface markers. This phenomenon may explain the presence of anti-fibroblast antibodies early in wound healing and the absence of immunologic activity in older wounds in which the keloid fibroblasts may reflect a more mature, nonantigenic and homogeneous, fibroblast population. Myofibroblasts are present in normal quantities in mature keloids (118, 119). Is the primary pathologic lesion in keloids attributable to AFA directed against and stimulating a susceptible fibroblast cell line to produce excessive amounts of collagen, or to an abnormal fibroblast population with an increased rate of collagen synthesis that present an antigenic challenge triggering the secondary appearance of AFA? Humoral immunity results classically in the synthesis of complement binding antibodies. The AFA are noncomplement fixing and may instead interact with nuclear proteins, as described previously.

Fibroblast heterogeneity may account for the abnormal fibroblasts that have been recovered from scar tissues. If the cell lines are atypical, the question arises as to why the lesions are limited to the skin, when fibroblasts are present throughout the body? Keloids are focal pathologic scars without systemic manifestations. A theory for the propensity for lesions to develop in specific anatomic locations has been proposed in the results of another study (81). The author of this study believes that secondary adaptations by fibroblasts to alterations in the regional environment may render them particularly sensitive to circulating substances. Fibroblasts from the reticular dermis synthesize more collagen in response to a circulating factor than those from the papillary dermis in patients with scleroderma (120, 121). Estrogen will cause different responses in collagen production by lung derived fibroblasts in comparison with dermis derived fibroblasts (122). Hypertrophic scar-derived fibroblasts demonstrated a decreased responsiveness to epidermal growth factor and tumor necrosis factor alpha and significantly elevated collagen synthesis in response to transforming growth factor beta when compared with normal cells (123).

Keloid fibroblasts, in vitro, exhibit a reduced dependence on serum growth factors similar to fetal cells reflecting an inappropriate expression of growth related genes (102). These data may indicate that the underlying mechanism responsible for fibroplasia may be identical in keloid and fetal wounds. Clinically, it is evident that keloid scars differ tremendously when compared with healing of fetal wounds, which is generally considered to be scarless. Clearly, the abnormal behavior of keloid fibroblasts is initiated and promoted by the environment of the wound. It is not known why the keloid wounds do not display the same degree of regulation of collagen deposition that fetal wounds exhibit. The "secondary adaptations" of keloid fibroblasts may also render them resistant to the typical negative feedback signals that normal cells obey. In another study, it was proposed that keloid fibroblasts may be unresponsive to normal feedback signals that regulate physiologic wound healing (52). The authors of the current article conducted a study that suggested that lymphocyte derived factors depress normal skin fibroblast collagen synthesis, whereas scar and keloid cells are progressively more resistant to the depressant effects. Thus,
potential explanations for the pathologic behavior and localized nature of keloids are an acquired susceptibility and resistance of regional fibroblasts to circulating substances, that is, inhibitory agents, immunologic mediators, growth factors or substances, limited to the wound site, which induce fibroblast transformation or a differentiated and localized fibroblast subpopulation that inherently produces excessive quantities of collagen.

SUMMARY

The mechanisms underlying the pathogenesis of keloids have not been fully characterized despite extensive past and present research. Results of past and present studies have shown that the immune system is actively involved in the development of these lesions. Future investigations into the biochemistry and immunologic factors of keloids are anticipated and expected to produce additional insight. The inability to identify cellular (fibroblast) abnormalities has led most investigators to focus on the humoral regulators of wound healing, that is, biochemical substances, immunologic mediators and growth factors. Future studies are needed to confirm or refute the presence of AFA. AFA, if they exist, may prove to be useful as immunologic markers of keloids and may help distinguish keloids from hypertrophic scar in the early stages of wound healing. The influence of immunologic mediators may be more impressive early in the development of scars. “Young” or “early” is defined as less than two years of age, whereas “old” or “late” keloids are more than two years of age. We suggest that future studies stratify keloids into early versus late and also measure the rates of collagen synthesis of fibroblasts derived from the normal and abnormal specimens from the same patient. Analysis of the leukocyte factors will clarify the role the immune system has in the regulation of collagen synthesis. Preliminary investigations have shown that immunotherapy may be of value in the treatment of keloids. The role of fibroblast heterogeneity needs to be investigated. It is not known which aspects of fibroblast heterogeneity are responsible for the localized and accelerated rates of collagen synthesis of keloid fibroblasts.

REFERENCES


