Collagen chain composition and collagen gene expression in acne keloids.

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Keloids are thick scars that grow beyond the boundaries of the original skin damage. Most lesions appear within one year of skin inflammation including burns, surgery and acne vulgaris. They are characterized by an excessive accumulation of extracellular matrix components. Keloid scarring results from increased activity of fibroblasts in the dermis with imbalance between synthesis and breakdown of collagen. Previous studies have indicated that collagen types I and III are the major components of the lesion [1]. Collagen types V and VI have also been located in keloid fibroblasts [2 and personal observations]. Conflicting results on collagen production within keloids have previously been reported [3, 4]. These reports cover a variety of lesions irrespective of the precipitating cause, but few have included acne keloids. The aim of this study was to investigate collagen composition and collagen gene expression in early and late lesions of acne keloids.

Six patients with untreated truncal keloids secondary to acne were studied. 1-3 month lesions were classified as early keloids (EK) and lesions that presented over 3 months were classified as late keloids (LK). 2-3 mm biopsies were obtained from patients under local anaesthetic using Lignocaine. Biopsies were cultured for 24 h at the air/liquid interface in methionine-free, modified Eagle's medium supplemented with 3% methionine. The biopsies were then washed, homogenised and lyophilised. Samples were treated with pepsin or with collagenase to degrade non-collagen or collagenous protein, respectively. Types I and III collagen were separated on 5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis in reducing conditions and autoradiographed. Immunoblot analysis was performed with anti-collagen type I (kindly provided by Dr J. Werkmeister, CSIRO, Australia) and type III (Lab. Impex Ltd. UK) antibody. Densitometric scans were used to quantify the collagen bands on autoradiographs.

In situ hybridisation was performed using a modified method of Lewis et al. [5]. Briefly, 3 mm cryosections were hybridised with biotinylated cDNA probes for procollagen α1(I), α2(I) and procollagen α1(III) (kindly provided by Dr G. Tromp and Dr Ala-Kokko, Philadelphia, USA). Sections were fixed in 4% formalin, treated with Nonidet + Triton X-100, proteinase K, acetic anhydride and post fixed in formalin. Hybridisation was performed at 37ºC for 24 h and RNA signal detected using the streptavidin/biotinylated alkaline phosphatase detection system.

Fig. 1 shows a typical result of immunoblotting of tissue homogenate with incubated with anti-collagen types I and III antibodies. Type III collagen was the predominant collagen in keloid tissue (Lane D). Reduced levels of type I collagen was seen in the tissue of normal appearance obtained from the border of the lesion (lane A) and keloid tissue (lane B).

Autoradiograph of EK tissue showed higher (6.5 ± 1.8 S.D.%) incorporation of radioactivity into α1(I) collagen than LK (3.8 ± 1.7%). Similar results were obtained in EK and LK for α2(I) collagen (6.8 ± 2.5% and 2.9 ± 0.9%, respectively) incorporation. EK showed over three-fold increase of radioactivity incorporation into α1(III) collagen compared with LK (11.0 ± 3% and 2.9 ± 1%, respectively).

High levels of pro α1(III) collagen cDNA-mRNA hybrids were detected in the dermis of early acne keloid lesions (Fig. 2). Expression of the pro α1(III) collagen gene was also noted in the periphery of blood vessels (arrows) within the keloid lesion. Lower levels of expression of pro α1(I) and pro α2(I) collagen were observed in the lesions, with relatively high levels of mRNA expression at the periphery of blood vessels. Increased collagen production is observed in early keloids compared with late keloids. Type III collagen synthesis and collagen gene expression was elevated in EK. Increased collagen gene expression was also seen in microvascular endothelial cells. Growth factors, e.g. transforming growth factor-β, platelet-derived growth factor, fibroblast growth factor are produced by neovascular endothelial cells in wound healing process. These growth factors may activate adjacent fibroblasts to express increased levels of collagen.

This work was supported by the Leeds Foundation for Dermatological Research.