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THE USE OF ATHYMIC NUDE MICE FOR THE STUDY OF HUMAN KELOIDS

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Abstract: Keloid tissue has been implanted in the athymic nude mouse in order to develop an experimental animal model for the study of human keloids and hypertrophic scars. Untreated keloid tissues maintained essentially the same morphological patterns and glycosaminoglycan distributions for at least 60 days after implantation in the athymic mice. Normal human skin implanted in the same way was maintained without change in glycosaminoglycan distribution or morphologic characteristics. We suggest that this model may be useful for basic research of keloids and hypertrophic scars in that it will allow studies of morphologic, biochemical and therapeutic interrelationships under controlled conditions.

Introduction: Basic research on keloids and hypertrophic scars has been greatly hampered because of the absence of suitable animal models for these conditions. Hypertrophic scars have been described in a number of farm animals; however, none of these have proven to be similar to human hypertrophic scars or keloids in regard to histology, biochemistry, or clinical history (1). Attempts have been made to induce these lesions in animals, particularly in swine (2). However, these scars have not been accepted as similar to human hypertrophic scars or keloids. Recently, it occurred to us that since normal and diseased human skin may be implanted and maintained in the athymic nude mouse (3,4,5,6) an animal model might be developed for keloid research by implanting keloids in athymic nude mice. Consequently, we implanted human keloid tissue in athymic nude mice and have investigagated whether the maintained tissue has the distinctive histological aspects and the biochemical composition of the original keloid tissue. Previous publications have described the characteristic microscopic appearance (7) and characteristic alterations of the glycosaminoglycans (8) in keloids and hypertrophic scars.

Materials and Methods: Athymic nude mice (NU/cox) were obtained from Laboratory Supply Company, Indianapolis, Indiana. They were kept in presterilized cages, one mouse to a cage, and placed in a laminar flow sterile bench (Labconco Corporation, Model 36000). They were fed sterilized Purina Mouse Chow, ad libitum, and given sterilized water to drink. Human keloid tissues were obtained from excess surgical material from the removal of keloid scar tissue from patients. In most cases, keloids had not been treated and were considered clinically active. Normal human facial skin was implanted in one set of animals. Implants of all tissues were made within four hours after removal from the patients. The tissues were refrigerated in sterile petri dishes between saline moistened gauze bandages until implanted.

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All procedures were carried out under aseptic conditions using sodium pentobarbital anesthesia. In general a 5-10 mm section of keloid or normal skin was de-epithelialized and inserted through a 1 cm incision into a subcutaneous pouch created between the shoulder blades; the incision was closed with a 11 mm wound clip. No dressings were applied to the mice, and they were returned to individual cages. No antibiotics were given to the animals. At various times, ranging from 7 to 60 days, mice were anesthetized with ether and the implants removed. The implant tissue was divided into two portions. One portion was placed in the deep freeze and later utilized for the glycosaminoglycan studies as previously described (8). This procedure consists of digestion of the skin samples with papain followed by separation of the glycosaminoglycans by cellulose acetate electrophoresis. The amount of each glycosaminoglycan fraction was estimated by means of a Beckman Microzone Densitometer and expressed as percentages of the total glycosaminoglycan levels. The other portion of tissue was fixed in Karnovsky's solution and processed for light microscopy. Paraffin blocks were sectioned at 6-8 μm and the section stained with hemotoxylin-eosin and by the Mason's trichrome method.

Results: For this study, eight different human keloids and one sample of normal facial skin were transplanted to nude athymic mice. In all, 37 keloid pieces were implanted and 4 normal skin samples were transplanted for lengths of time varying from 7 to 60 days. None of the samples were rejected or became necrotic. Examination by light microscopy indicates that all of the implanted tissues, normal and keloid, retained their viability and original histological character.

Figure 1 shows a section of a keloid with typical nodules prior to transplantation. Figure 2 demonstrates that tissue from the same keloid after implantation for 27 days has retained the typical keloid structure. There is no cell degeneration and no inflammatory cell infil-

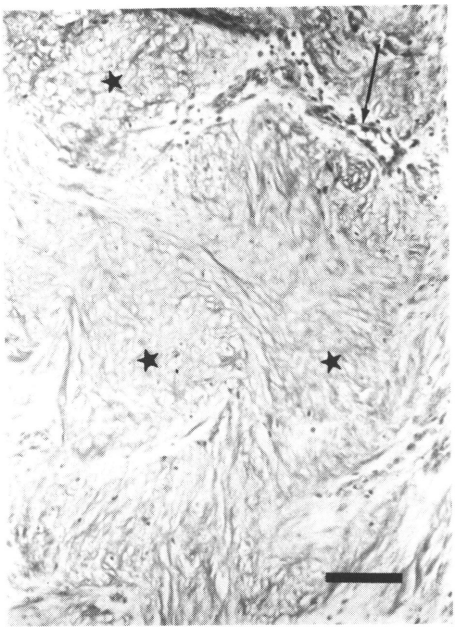

Figure 1. Section of Keloid #1 before implantation. Nodules are cut in cross section (★). Note: Perivascular vessels (⟶). Scale bar = 100 μm. (x125)

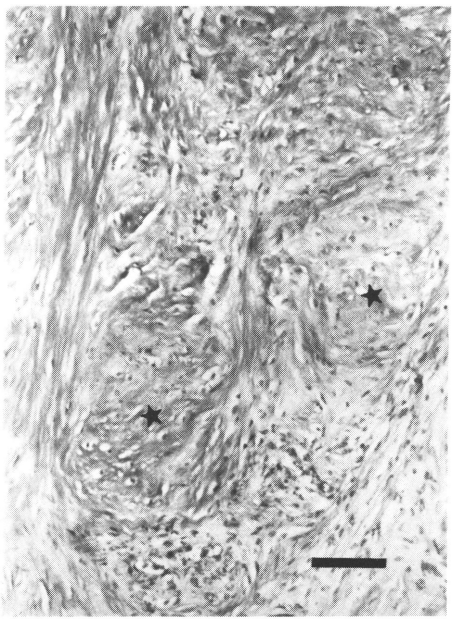

Figure 2. Section of Keloid #1 after maintenance in athymic nude mouse for 27 days. Note: Nodules (★) similar to Figure 1. Scale bar = 100 μm. (x125)

Table I

Glycosaminoglycan Distribution in Keloid Tissue and Normal Skin Before and After Implantation in Athymic Nude Mice

	Percent of Total Glycosaminoglycan		
	Hyaluronic Acid	Chondroitin-4-Sulfate	Dermatan Sulfate
Original Keloid*	23.7 ± 3.6	21.8 ± 2.3	54.5 ± 4.2
Implant*	25.0 ± 3.4	19.2 ± 1.8	55.8 ± 3.3
Original Keloid**2A	22.0	26.0	52.0
Implant 20 days	19.0	25.0	56.0
Implant 36 days	15.0	24.0	61.0
Implant 60 days	15.0	24.0	61.0
Normal Skin	44.2	5.0	50.8
Implant 40 days	43.2	4.5	52.3

* Average of seven different active keloid tissues before and after implantation for 20-36 days. Figures following ± are standard errors of the mean. The results were analyzed statistically by Student's t Test. None of the differences between the original keloid and the implants were statistically significant.
** Portions of one active keloid were implanted in different animals and removed at the times indicated.

tration. Further, the implants apparently revascularize, as many sections of vessels contain erythrocytes.

The average distribution of glycosaminoglycans in the original active keloid tissues and in the corresponding implanted tissues is summarized in Table I. The chondroitin-4-sulfate levels of the original keloid tissues varied between 10.7 and 28.0%, while the hyaluronic acid levels varied between 13.1 and 40.0% of the total glycosaminoglycans. Based on our previous research, these glycosaminoglycan distributions are typical of actively proliferating keloids with increased chondroitin-4-sulfate and decreased hyaluronic acid (8). The histological appearance agrees with these data. The table also includes data for one normal facial skin sample implanted and removed after 40 days. The glycosaminoglycan distribution was unchanged in this implant.

Discussion: This preliminary study indicates that the athymic nude mouse may be used to provide an experimental animal model for studies of hypertrophic scars and keloids. Of the eight keloids implanted, all were maintained with preservation of the original morphological and glycosaminoglycan distribution for the times studied after implantation. Normal skin was maintained in the athymic mice without change in morphology and glycosaminoglycan composition. Further biochemical and morphologic studies should be of interest and are currently under way. It appears that implants of keloid tissue in athymic mice will provide an animal model for studies of keloids and hypertrophic scars under controlled conditions.

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