PERIVASCULAR MYOFIBROBLASTS AND MICROVASCULAR OCCLUSION IN HYPERTROPHIC SCARS AND KELOIDS

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Microvessels in normal skin, granulation tissue, hypertrophic scar, keloid, and mature scar from human subjects were studied by transmission electron microscopy. Comparative observations suggested that most microvessels in hypertrophic scar and keloid are occluded or partially occluded, apparently owing to an excess of endothelial cells. Endothelial cell contraction was also supported by the observations, and perivascular satellite cells (pericytes), some of which were identified as myofibroblasts, were observed in hypertrophic scars and keloids. Among findings from statistical analyses were that 1) the patency of microvessels in hypertrophic scar and granulation tissue is similar, as is that of microvessels in keloid and mature scar, but the patency of all these microvessels is significantly less than that of microvessels in normal skin, and 2) endothelial cell density is greater in nonpatent vessels than in patent vessels. The observed extent of microvascular occlusion supports a previously published theory that hypoxia is involved in the generation of hypertrophic scar. Hum Pathol 13:819–824, 1982.

In one of our early studies of hypertrophic scar we observed microvessels (capillaries) with occluded lumens.[1] This phenomenon was observed again in later studies.[2–4] The occlusion appeared to be the result of an excessive number of endothelial cells that bulged into the lumen. When we suggested in an earlier publication that chronic hypoxia might be involved in the development of hypertrophic scar,[2] the possibility of extensive microvascular occlusion assumed greater significance, since such a condition could account for the suspected hypoxia. Recently, Sloan et al.[5] verified hypoxia in hypertrophic scar.

For the past two years we have deliberately screened every sample available to us of hypertrophic scar, keloid, mature scar, and granulation tissue for ultrastructural characterization of the microvessels. In addition, we have secured and screened samples of normal skin. This paper reports an assessment of the microvessels for each group as determined by statistical analysis.

MATERIALS AND METHODS

Human tissues were obtained from surgical excision procedures. The tissue samples included 11 of normal skins, 13 of hypertrophic scar, nine of keloid, five of mature scar, and nine of granulation tissue.

These tissues were dissected appropriately, fixed immediately in Karnovsky's solution, and stored from two hours to several days. Cores 1 mm in diameter were obtained by means of a modified Jahmshidi liver punch and washed in isotonic cacodylate buffer at *p*H 7.4. The tissues were then postfixed in isotonic osmium tetroxide. After ethanolic dehydration, the tissues were embedded in Epon 812. Thick sections were cut at 1 μm and stained with toluidine blue. Thin sections were stained with uranyl acetate and examined in a Philips 300 transmission electron microscope.

These tissues were examined primarily for evaluation of the microvasculature. The diameters of the microvessels ranged from 3.3 to 14.6 μm, which is the normal range. Rarely was any larger vessel encountered in any tissue group, except normal skin. The ultrastructural characterization of the microvessels included determination of 1) luminal patency (or occlusion), 2) the numbers of endothelial cell and nuclear profiles, 3) contraction of endothelial cells, determined largely by endothelial nuclear crenation, and 4) types and conditions of perivascular satellite cells (pericytes). The following numbers of microvessels were studied in each group: 25 in normal skin, 76 in granulation tissue, 306 in hypertrophic scar, 106 in keloid, and 51 in mature scar. Only microvessels cut unambiguously in cross-sections (usually round in profile) were used in the data collection. Only a rare view of cross-sectioned microvessel suggested collapse of the wall. No confusion existed in distinguishing vessel collapse from noncollapse. Full occlusion was based on a lumen less than 1 μm in diameter. Partial occlusion was assessed for lumens up to 3 μm across; beyond that the microvessel was considered to be patent.

The patency data were examined using chi-square statistics determined from contingency tables. In addition, 95 per cent confidence intervals were determined for each tissue group. Endothelial cell density was assessed across tissue groups and was compared between patent and nonpatent vessels using a two-way analysis of variance.

Further characterizations were based on comparative quantities in the endothelial cells of 1) microfilaments, 2) Weibel-Palade bodies, 3) microvesicular bodies, 4) mitochondria, 5) Golgi membranes, and 6) rough endoplasmic reticulum. Gradings on

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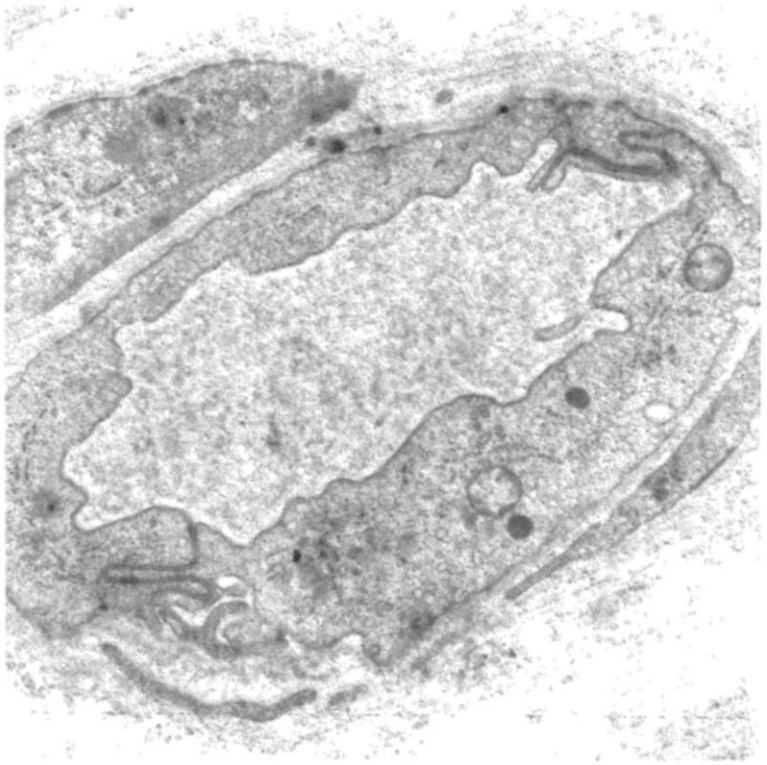

Figure 1. Capillary from dermis of normal skin. Two endothelial cells are evident and cell junctions are simple. Endothelial cytoplasm contains few organelles but many micropinocytotic vesicles on both luminal and abluminal sides. Sections of a likely single pericyte are present with essentially the same void of organelles as the endothelium. (×19,500.)

a scale of 0 to 5 were assigned arbitrarily from study of electron micrographs.

RESULTS

Electron Microscopy

Figure 1 illustrates the typical fine structure of a capillary from normal skin. In this section, profiles of apparently two endothelial cells can be seen. The junctions of the endothelial cells are relatively simple.

Endothelial cytoplasm contains a few microfilaments, a scattering of free ribosomes, an occasional profile of rough endoplasmic reticulum, but an abundance of micropinocytotic vesicles on both the luminal and abluminal sides. Underlying the endothelium is a simple basal lamina comprising granular and short filamentous structures approximately 30 nm thick. Outside this layer but encased in their own basal lamina are the pericytes. In any given cross-section, a profile of usually just one pericyte cell will be seen. The cells closely resemble endothelial cells, although we have never observed Weibel-Palade bodies in them.

Occluded capillaries were sometimes observed in normal skin. Usually these occurred in so-called normal samples taken adjacent to hypertrophic scar lesions.

Occluded capillaries predominated in hypertrophic scar and keloid. Only occasionally were patent vessels observed in this group. Figure 2 shows a cross-section of a capillary representative of those in hypertrophic scar. It is 8 μm in diameter. Observed in this section are profiles of at least 12 endothelial cells, including three endothelial nuclei. The lumen is occluded. Note also the high position of the tight junctions between the endothelial cells. There are a few organelles within the endothelial cytoplasm, but, although not seen at the magnification of figure 2, the cytoplasm is rich in microfilaments. Endothelial nuclei are essentially round. Surrounding the endothelium is a single layer of basal lamina. In most cases, however, the basal lamina is thickened and multi-layered.

Also seen in figure 2, a satellite cell nearly circumscribes the entire periphery of the endothelium. The inner rim of this satellite cell displays a band of microfilaments that is aligned parallel to the circumference of the vessel and periodically shows cytoplasmic densities (*arrows*). Such a band is never seen at the periphery of a cell. No similar satellite cells have been observed in normal dermis or mature scar. We interpret these pericytes as myofibroblasts.

In other occluded microvessels, endothelial nuclei appeared highly lobated or crenated. In figure 3, five profiles of endothelial cells can be seen. Outside this layer is a complete basal lamina. The satellite cell again shows the inner band of microfilaments and cytoplasmic densities. Outside the satellite cell is a multilayered basal lamina.

Complex unions between endothelial cells were often observed (fig. 4). Despite this, tight junctions or gap junctions were virtually always seen and in most instances were positioned high on the cell boundaries. No wide-open breaks or gaps have yet been observed.

The endothelial cells in hypertrophic scars usually contain notable amounts of microfilaments, increased numbers of mitochondria, Golgi membranes,

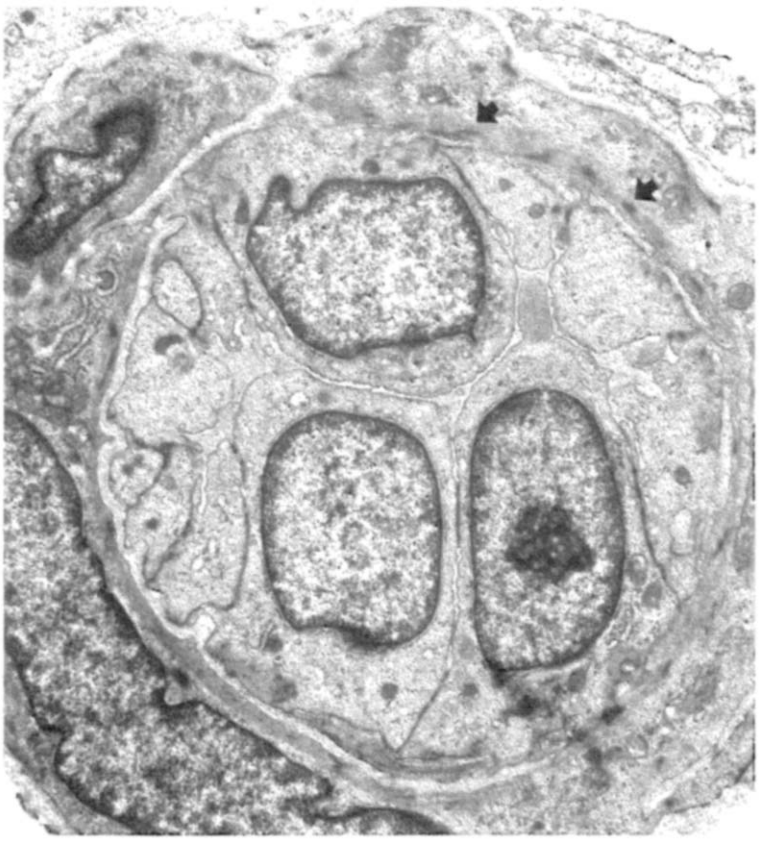

Figure 2. Cross-section of microvessel from hypertrophic scar. Profiles of at least 12 endothelial cells are present. A satellite cell is present with an inner rim of microfilaments and cytoplasmic densities (*arrows*). Note also the high position of interendothelial tight junctions. (×10,200.)

and profiles of rough endoplasmic reticulum. The numbers of Weibel-Palade bodies and multi-vesicular bodies did not appear to differ from those in all other groups, including normal dermis. Although vesicles were not counted, we observed that there are usually fewer micropinocytotic vesicles in endothelial and satellite cells of hypertrophic scar and keloid compared with normal dermis.

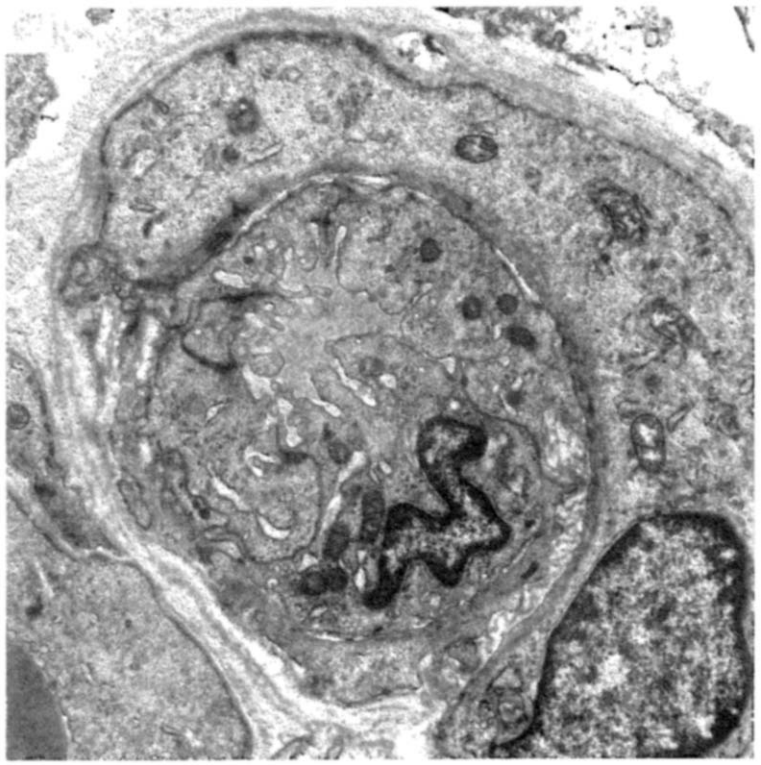

Figure 3. Partially occluded microvessel from hypertrophic scar. Note crenated endothelial nucleus. Five endothelial cells are present. The satellite cell shows an inner rim of microfilaments and cytoplasmic densities. The basal lamina is complex and thickened. (×10,200.)

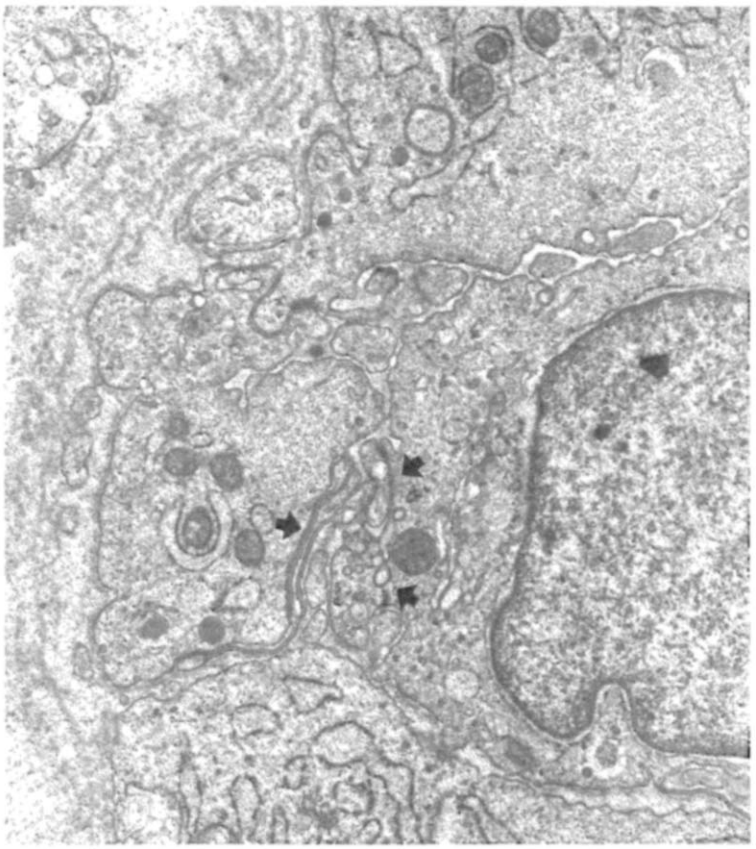

Figure 4. Complex endothelial junctions in occluded vessel from hypertrophic scar (*arrows*). (×18,000.)

Statistical Analysis

The diameter of the microvessels in the tissues examined varied from 3 to 15 μm, but the majority were between 7 and 9 μms. Table 1 shows the means and ranges of diameters for all vessels studied from the several groups. All were of equivalent size.

The results of chi-square statistical analyses of the patency frequencies for all groups are shown in table 2. Patency rates were approximately 60 per cent for normal skin, 7 per cent for granulation tissue and hypertrophic scars, 17 per cent for keloid, and 20 per cent for mature scar. Chi square analysis of the data shown in table 2 indicated statistical significance ($P < 0.001$).

Examining a 95 per cent confidence interval for patency across tissue groups, we confirmed that granulation tissue is similar to hypertrophic scar and that keloid is similar to mature scar. Each group indicated a patency rate significantly lower than that of normal skin.

Table 3 shows the density of endothelial cells in all microvessel profiles observed. A two-way analysis of variance using the number of endothelial cell profiles per square micrometer yielded the following results: 1) patent vessels showed significantly less endothelial density than nonpatent vessels ($P = 0.01$); 2) controlling for patency, we found no significant differences among the tissue groups ($P = 0.13$); and 3) there was no significant interaction between patency and tissue group ($P = 0.89$).

Analysis of variance data for the numbers of nuclear profiles indicated no significant differences among the tissue groups studied.

DISCUSSION

The vast majority of the microvessels in the hypertrophic scars and keloids had occluded lumens. This finding contrasted sharply with that of microvessels observed in normal skin. The fact that occluded microvessels were sometimes observed in some of our normal samples may reflect our inability to obtain truly normal skin samples. Most of our specimens were from areas immediately adjacent to a lesion or were obtained from lipectomies.

The microvessels of the hypertrophic scars and of many keloids very often appeared to have several satellite cells about them. This contrasted with what is usually observed for a capillary, where one perivas-

TABLE 1. DIAMETERS OF MICROVESSELS (μm)

		Mean ± SD	Minimum	Maximum
Normal skin	(n = 25)	8.2 ± 2.7	3.3	13.9
Granulation tissue	(n = 76)	9.2 ± 2.3	4.5	13.8
Hypertrophic scar	(n = 306)	8.3 ± 2.3	3.3	14.6
Keloid	(n = 106)	7.8 ± 2.4	3.7	14.5
Mature scar	(n = 51)	7.7 ± 2.2	3.8	13.3

TABLE 2. PATENCY OF MICROVESSELS*

		Patency (%)	Partial Occlusion (%)	Full Occlusion (%)	95% Confidence Interval for Patency (%)‡	
					Low	*High*
Normal skin	(n = 25)	60	36	4	39	79
Granulation tissue	(n = 75†)	6.6	30.7	62.7	2	15
Hypertrophic scar	(n = 306)	6.9	19.3	73.8	4	11
Keloid	(n = 105†)	17.1	20.0	62.9	11	26
Mature scar	(n = 51)	19.6	33.3	47.1	10	33

* $\chi^2 = 51.7, P < 0.001$ (see text for discussion).
† Data not determined for one case.
‡ Documenta, Geigy, Scientific Tables, 6th Edition, page 86, 1962.

TABLE 3. ENDOTHELIAL CELL DENSITY (per μm^2)*

	No. of Vessels		Mean density (±SD)		Minimal Density		Maximal Density	
	Patent	*Occluded*†	*Patent*	*Occluded*	*Patent*	*Occluded*	*Patent*	*Occluded*
Normal skin	15	10	0.09 ± 0.04	0.13 ± 0.05	0.05	0.07	0.15	0.23
Granulation tissue‡	5	70	0.07 ± 0.03	0.10 ± 0.05	0.04	0.03	0.11	0.29
Hypertrophic scar	21	285	0.10 ± 0.05	0.14 ± 0.07	0.03	0.03	0.19	0.53
Keloid	18	87	0.10 ± 0.05	0.14 ± 0.09	0.03	0.03	0.24	0.52
Mature scar	10	40	0.13 ± 0.05	0.14 ± 0.07	0.08	0.05	0.26	0.33

* Results of two-way analysis of variance: effect of patency significant ($P = 0.01$); effect of tissue not significant ($P = 0.13$); no interaction between patency and tissue ($P = 0.89$). (See text for further discussion.)
† Includes partial and full occlusion.
‡ Data not available for one case.

cular satellite cell or none may be present.[6]. In many cases the innermost satellite cell had every characteristic of a myofibroblast. In any event, no previous report known to us identifies a myofibroblast as a pericyte of capillaries.

The occlusion we report may be due to any of three possible mechanisms, or combinations of these: 1) perivascular myofibroblast contraction, 2) endothelial cell contraction, or 3) endothelial cell proliferation.

Pericapillary myofibroblasts were observed in about half of the hypertrophic scars and keloids we studied. It may be that they eventually migrate from the perivascular position to the interstitial area, where they are much more numerous. After a time they may be transformed into fibroblasts. Pericapillary satellite cells are suspected of being the source of new fibroblasts in dermal wounds[7–9] and, possibly, liver cirrhosis.[10] Indeed, our first study of granulation tissue showed a plethora of pericytes about the newly growing capillaries.[3] The smooth contour of nuclear sections through the pericapillary myofibroblast is not considered equivocal to a proposed active contraction, since only the inner or vascular side of the cytoplasm contains the microfilaments and densities. Furthermore, active contraction of the myofibroblast, which would have a direct functional effect on the microvessel, is suggested because the microfilaments have not been observed elsewhere in the cell.

Evidence of endothelial cell contraction, which might contribute to luminal occlusion, has not been consistently observed. Crenated nuclei are the exception, not the rule. Although endothelial cell contraction, as previously reported,[11–13] would tend to shorten a vascular segment, a bulging of the nuclear region could protrude into the lumen, thereby effecting an occlusion. However, we have found no consistent evidence of shortened vascular segments by multiple light microscopy studies of serial paraffin sections.

By far the most plausible mechanism for occlusion can be supported by the density of profiles of endothelial cells observed in cross-sections. The endothelial cell density data also strongly argue against occlusion due to collapse. Besides, collapse is not really tenable in light of round profiles of microvessels. Furthermore, figure 2, in which a microvessel 8 μm in diameter is occluded, showing profiles of 12 endothelial cells, also demonstrates tight endothelial junctions situated high between endothelial cells. This was a common observation; it would not occur if the endothelial cell density increased at a given focus of the vessel owing to either contraction or endothelial cell collapse. It also is not reasonable to suspect that the occluded microvessels in hypertrophic scar and keloid are simply the terminal closed ends of growing vessel buds. In hypertrophic scars and keloids of some years' standing, this activity, expected in granulation tissue, would have long since ceased.

Occlusion is essentially the same process in each group, as judged from the interaction statistic. The density data also suggest that some normal cases were probably not normal. Indeed, some of those cases were probably "normal" tissue immediately adjacent to the scar or keloid lesion.

It is likely that endothelial cell proliferation is the major factor contributing to occlusion. However, it is also likely that perivascular myofibroblast and endothelial cell contractions also contribute, albeit minimally.

The ultrastructure of most endothelial cells in hypertrophic scar and keloid is in concert with an active biologic state. Whether this indicates proliferation cannot be definitely stated here. However, proliferation could be supported by increased numbers of organelles and many intercellular junctions that form complex unions or mazes of intricate partially interdigitated cell processes.

It is of special significance that in our previous study of a first series of granulation tissues from deep human wounds[3] we found occluded microvessels only in older granulations, those in which the collagen patterns of hypertrophic nodules had already been well established. Additionally, fibrin polymer was persistent in every case examined.

It appears likely that microvascular occlusion in hypertrophic scars and keloids is due principally to increased numbers of endothelial cells. It may also be that a proliferation is established in the granulation phase. The factors promoting a suspected proliferation are not known; however, because we have observed pervasive and persistent fibrin in our series of granulation tissues, we suggest that this is a possible source of stimulation. Fibrin has been identified as stimulating a marked uptake of ^{3}H-labeled thymidine in cultures of fibroblasts.[14,15]

Once again, we are compelled to reaffirm our hypthesis that hypoxia is an integral factor in the generation of hypertrophic scar and keloid. We have provided strong morphologic evidence of the origin of the hypoxia. Whether the hypoxia may stimulate proliferation of endothelial cells, pericytes, and fibroblasts or the synthesis of collagen, or all, remains to be determined. Hunt et al.[16] argue convincingly for hypoxia controlling collagen synthesis in the wounds of rabbits and dogs.

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