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Microcirculatory Segments Identified with Monoclonal Antibody against α-smooth Muscle Actin: Comparison between Kimura's Disease and Angiolymphoid Hyperplasia with Eosinophilia

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Microcirculatory systems, which can be divided into several segments, have crucial physiological functions. We investigated whether monoclonal antibody against α-smooth muscle actin (αSMA) was useful for the identification of microcirculatory segments, according to the structure of their periendothelial cells, in two pathological cutaneous conditions. We examined skin specimens from patients with Kimura's disease and with angiolymphoid hyperplasia with eosinophilia, since little information is available on microvascular segments in these conditions. Immunostaining for αSMA revealed the morphological characteristics of the periendothelial cells clearly enough to identify five segments in the proliferative microvessels of Kimura's disease and angiolymphoid hyperplasia with eosinophilia. In Kimura's disease, postcapillary venules were predominant, while each vascular segment in angiolymphoid hyperplasia with eosinophilia was uniform. Vessels without periendothelial cells were detected to a greater extent in angiolymphoid hyperplasia with eosinophilia than in Kimura's disease. The antibody against αSMA appeared to be useful in the observation of periendothelial cells for the identification of vascular segments in pathological cutaneous conditions. ***Key words: microcirculatory system; postcapillary venule; periendothelial cell.***

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The microcirculatory systems between arterioles and venules carry out crucial physiological functions in vertebrates, such as blood-flow regulation and exchange processes. Some authors (1–5) have divided these systems into several different segments based on functional and structural differences by light microscopy and transmission electron microscopy. However, they did not clearly show the three-dimensional morphological characteristics of the microvascular walls and the periendothelial cells in each segment. It has therefore been difficult to identify the segment to which a microvessel belongs from the morphological findings. Fujiwara & Uehara (6) have since then demonstrated the three-dimensional structure of periendothelial cells (PEC) in microcirculatory beds, using scanning electron microscopy. Their excellent photographs have enabled us to classify the microvascular segments based on the morphological differences of the PEC as: terminal arterioles, metarterioles, true capillaries, postcapillary venules and muscular venules. However, this requires a complicated technique and consequently seems to be unsuitable for studies of many cutaneous pathological conditions.

Recently, a monoclonal antibody against α-smooth muscle actin (αSMA) was shown to be an extremely useful and reliable probe for smooth muscle cells (7), with which the PEC were positively stained (8). Hirano et al. (9, 10) showed that immunohistochemical staining for αSMA revealed the morphological characteristics of the PEC clearly enough to identify each vascular segment in normal human skin tissue by light microscopy. They concluded that the antibody against αSMA was superior to that against desmin in demonstrating PEC. These findings suggest that the identification of microcirculatory segments with this antibody would be helpful in understanding skin disorders, if suitable for use in pathological cutaneous conditions.

In this study, we investigated whether immunostaining for αSMA showed the morphological characteristics of PEC clearly enough to allow the identification of vascular segments in some pathological cutaneous conditions as well as those in normal skin tissue. For this purpose, we examined skin specimens obtained from patients with the vascular proliferative disorders Kimura's disease and angiolymphoid hyperplasia with eosinophilia (ALHE). Although the clinical and pathological distinctions between the two entities have been elucidated (11–15), little information is available on the differences in the composition of microvascular segments. Accordingly, we also compared the composition of the microvascular segments in these two conditions.

MATERIALS AND METHODS

Specimens from 4 patients with Kimura's disease and 3 with ALHE diagnosed by us between 1986 and 1991 were included in this study (Table I); all met the histological criteria of earlier studies (11–15). Biopsy specimens of the skin were fixed with 20% formalin and embedded in paraffin. Sections for immunohistochemistry, 20 mm, were then serially cut with a microtome, followed by deparaffinization with xylenes and rehydration in a graded series of alcohol. Immunohistochemical procedures were performed, using an avidin-biotin-peroxidase complex (ABC) technique as previously described (16). Commercially available primary antibody against αSMA (clone 1A4, Bio Makor, Israel), Ulex europaeus-1-biotin (UEA-1, DAKO, USA) and an ABC Vectastain kit (Vector Laboratories, USA) were used. Double-staining for αSMA and UEA-1 was also carried out, in which the positive reaction for UEA-1 was visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., USA) and the color of staining for αSMA was developed with DAB and nickel ammonium sulfate (Sigma Chemical CO.). Morphological identification of each microcirculatory segment was based on the detailed information on the overall shape and arrangement of PEC detected with scanning electron microscopy (6).

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Table I. *Summary of clinicopathological findings*

	Case/ Sex	Age (years)	Location	Size (cm)	Duration	Histological location
	1	38/M	Thigh	5	6 years	Deep subcutis
Kimura's	2	48/M	Lumbar	12	3 years	Subcutis
disease	3	35/M	Parotid	3	7 years	Deep subcutis
	4	52/M	Parotid	6.5	2 years	Deep subcutis
	5	48/M	Preauricular area	1	3 months	Dermis and superficial subcutis
ALHE	6	34/M	Scalp	0.5–1	4 months	Dermis and superficial subcutis
	7	28/F	Postauricular area	1	6 months	Dermis

RESULTS

In all materials, immunoperoxidase staining revealed that PEC were strongly positive for αSMA and that endothelial cells were positive for UEA-1. When the microvessels were cut lengthwise on glass slides (such vessels constituted about 20% of all vessels), the morphological characteristics of the PEC were detected clearly enough to make possible the classification of their microcirculatory vessels into five segments, corresponding to those detected by scanning electron microscopy (6) as: terminal arterioles, metarterioles, true capillaries, postcapillary venules and muscular venules (Fig. 1A–E). The findings are consistent with those in normal human cutaneous tissue (9, 10). Fig. 2 shows a schematic illustration of these findings.

Staining with UEA-1 revealed many vessels throughout the lesion in both Kimura's disease and ALHE (Figs. 3, 4), although there were fewer proliferative vessels in Kimura's disease than in ALHE. Cells positive for αSMA were detected only around the vascular lumina in both of them. Proliferative vessels were dilated variably, branched and/or anastomosed in ALHE. In Kimura's disease, most of the vessels were located between the lymphoid follicles, some of them flowing into the lymphoid follicles. In ALHE, all microcirculatory segments were equally identified among the proliferative vessels as in normal human tissue (data not shown), whereas in Kimura's disease, postcapillary venules with characteristic lacework spidery pericytes were conspicuous. There was no relation between the location of the proliferative vessels and the segmental component. However, as noted above, identification of segments was possible when the microvessels were cut longitudinally, and thus the results were not suitable for quantitative analysis. Dual staining for UEA-1 and αSMA showed many vessels lacking PEC in ALHE (Fig. 5) but only a few vessels without any pericytic investments in Kimura's disease, as in normal human tissue (data not shown). A few arterio-venous shunts were recognized in both.

DISCUSSION

It is customary to classify microcirculatory segments according to the caliber of the vessel. However, this subdivision is often unsatisfactory, as the caliber and structure of the vessel wall cannot always be correlated (17). Identification of the segment by the vessel wall structure is also unreliable, since the wall structure of venules is not very different from that of capillaries (17). Identification of the segments by the structure of the PEC is, however, useful and reliable, as the PEC in each segment have a characteristic morphological appearance. Our present study showed that the morphological findings of PEC detected with the monoclonal antibody against αSMA corresponded well to those observed by scanning electron microscopy and were clear enough to identify the microcirculatory segments in two cutaneous vascular proliferative disorders, Kimura's disease and

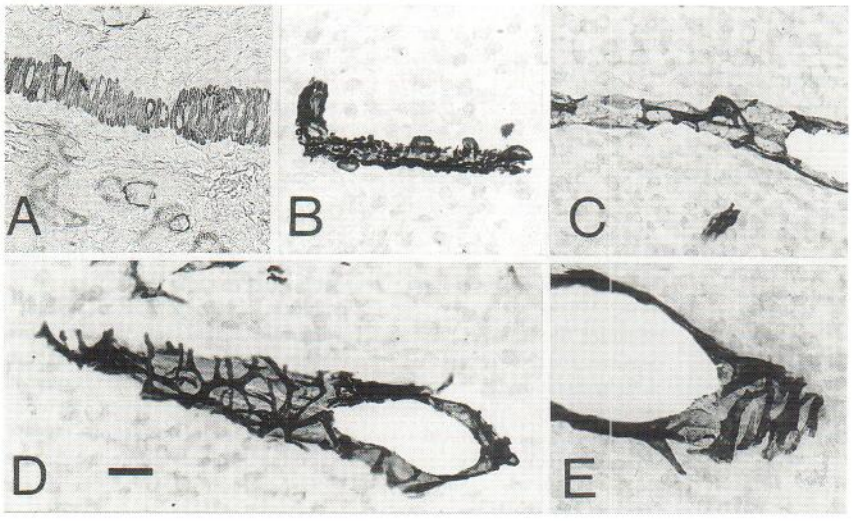

Fig. 1A–E. αSMA-positive microvascular periendothelial cells. A. Terminal arterioles: band-like unbranched vascular smooth muscle cells are surrounded in a circular fashion. Adjacent PEC are close to each other; the gap between is narrow. B. Metarterioles: round cell bodies are characteristic. The gaps between the neighboring cells are wider than those of terminal arterioles. C. True capillaries: individual PEC have round or hemispheric cell bodies with longitudinal primary processes and circumferentially oriented secondary processes. Large parts of the surfaces of the vessels lacked pericytic investment. D. Postcapillary venules: the PEC have more ramifying processes than those of true capillaries, which overlap and/or attach to each other in a meshwork fashion. E. Muscular venules: the vessels are surrounded in a circular fashion by relatively wide, ribbon-like PEC. Intercellular gaps are broader than those of terminal arterioles. ABC technique (A, D: Kimura's disease, B, C, E: ALHE). *Bar*, 20 μm. A–E × 300.

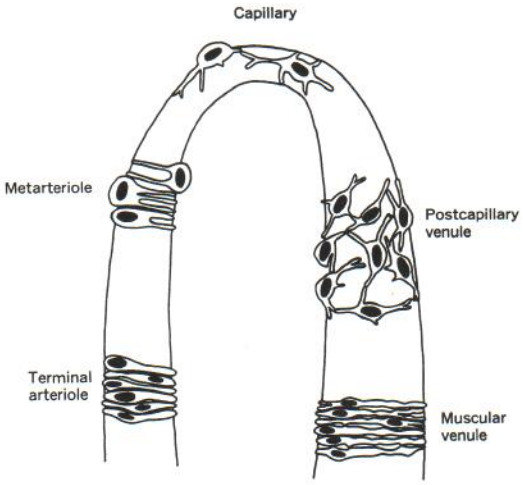

Fig. 2. Schematic illustration of periendothelial cells in human microvascular system.

ALHE, as well as in normal skin tissue. Moreover, the antibody against αSMA enabled us to observe PEC by conventional immunohistochemical methods, using paraffin-embedded specimens, whereas scanning electron microscopy requires much time and the use of fresh samples, of which supplies are limited. The monoclonal antibody against αSMA thus seems to be very useful for studying the microvascular system in certain cutaneous pathological conditions. For example: On what segment of the microvessels are cellular adhesion molecules mainly expressed in various inflammatory skin diseases? Which of the segments are chiefly involved in cutaneous vasculitis? Is there any relationship between certain microcirculatory segments and nerve endings with specific neuropeptides?

Attempting to analyze the composition of microcirculatory segments quantitatively, we encounter the problem that the segments are identified when the microvessels are cut lengthwise. We expect that observing much thicker slices will overcome this problem; it will therefore be necessary to improve the permeability of the antibody. Another problem in quantitative

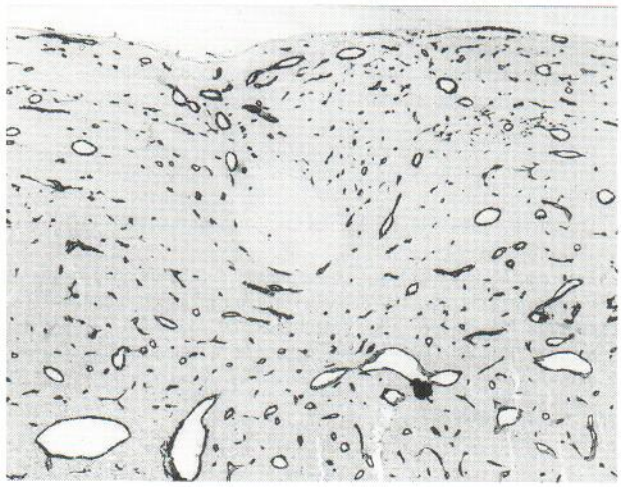

Fig. 3. Many vessels positively stained for UEA-1 are evident throughout the dermis in ALHE. ×40.

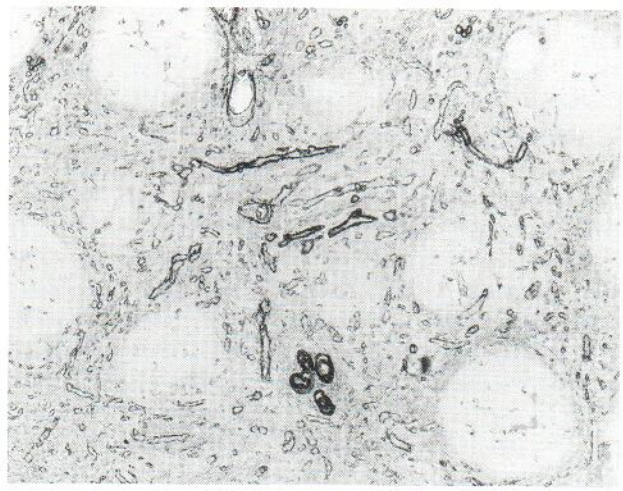

Fig. 4. Many vessels are located between the lymphoid follicles in Kimura's disease. ABC technique for αSMA. ×40.

analysis is the way we should assess segments in the intersegmental transitional region. With regard to the transitional region, a definite criterion is required as to whether this region should be identified and analyzed as a transitional region or whether only the distinct segments should be analyzed.

Kimura's disease is an uncommon chronic inflammatory disorder, first described by Kimura et al. in 1948 (18). Many reports have since followed, mainly in Japan and other Oriental countries. The histological features are characterized by variable degrees of vascular proliferation and polymorphic infiltrations, consisting of eosinophils and lymphocytes, with the formation of lymphoid follicles. Mehregan & Shapiro (19) subsequently introduced the term ALHE for a similar histological condition. It had been controversial for a decade whether these two disorders, Kimura's disease and ALHE, were identical, since they exhibit such histopathological similarities as vascular proliferation and

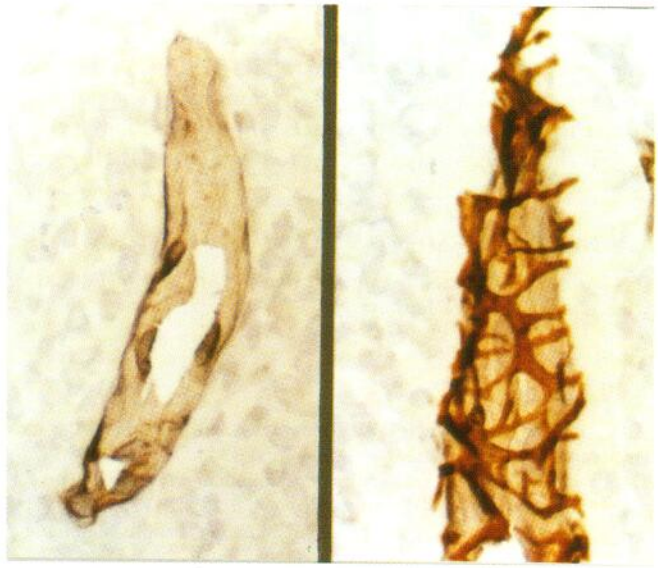

Fig. 5. Left, vessel lacking αSMA-positive pericytic investment; *right*, vessel with lacework pericytes stained positively for αSMA. ABC technique, dual stain for αSMA (brown) and UEA-1 (pale blue) in ALHE. ×40.

tissue eosinophilia. However, many comparative clinicopathological studies have since established differences between the two entities (11–15), and it is now possible to make a differential diagnosis, both clinically and histologically.

Although the morphology of the endothelial cells in the proliferative vessels in Kimura's disease and ALHE has been studied extensively, the segmental components of their proliferative vessels have been discussed, to our knowledge, in only two reports. From the histological findings, using a transmission electron microscope, Eady & Wilson Jones (20) suggested that the proliferative vessels in pseudopyogenic granuloma, included in the entity of ALHE, consisted mainly of postcapillary venules. Googe et al. (11), who observed the morphological features of the endothelial cells in two conditions, measured the caliber of the proliferative vessels, and found that postcapillary venules were conspicuous in Kimura's disease, while the vessels in ALHE were too abnormal to be classified. In this study, we made use of an antibody against αSMA to analyze the components of the microcirculatory segments of the proliferative vessels in Kimura's disease and ALHE. In Kimura's disease, we detected considerably more postcapillary venules than other segments. These findings were consistent with those of Googe and co-workers (11). On the other hand, we found that the proliferative vessels in ALHE consisted of all segments almost uniformly. Although the results could not be analyzed quantitatively, our observations suggest that the segmental components of proliferative vessels in Kimura's disease and ALHE are different.

The absence of pericytes has been correlated with the onset of neovascularization (21). Before the beginning of the new vessel growth associated with diabetes mellitus, there is a loss of pericytes, termed "pericyte drop-out" (22). Hemangiomas with excessive endothelial cell proliferation have few, if any, PEC (23). Orlidge and associates (24) showed that, in vitro, PEC had the capacity to inhibit endothelial cell growth via a mechanism that required contact or proximity between them, with transforming growth factor β acting as a mediator. We found here that many proliferative vessels in ALHE lacked pericytic investment, while few vessels without PEC were found in normal cutaneous tissue (unpublished observations) or in Kimura's disease. The presence of many vessels without PEC may help to explain the mechanism of vessel proliferation in ALHE.

ACKNOWLEDGEMENT

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