Three-Dimensional Reconstruction of Endothelial Cell Gaps in Psoriatic Vessels and Their Morphologic Identity with Gaps Produced by the Intradermal Injection of Histamine

Irwin M. Braverman, M.D. and Agnes Keh-Yen, B.S., M.S.
Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Endothelial cell gaps in psoriatic vessels and histamine-induced gaps in forearm skin of normal controls were reconstructed in 3 dimensions by a computer graphics system. The gaps in psoriatic vessels were present within the cell, at the intercellular junction, or concurrently at both sites. Histamine-induced gaps were found at the intercellular junction or at both intracellular and intercellular locations. The gaps were linear to oval and often contained cytoplasmic processes from one of the endothelial cells, suggesting that gap formation represents a cellular injury rather than a purely physiologic reversible phenomenon. J Invest Dermatol 86:577–581, 1986

In previous reports [1,2] we described endothelial cell gaps in postcapillary venules and, less commonly, in the venous capillary loops of psoriatic plaques and patches of pustular psoriasis (von Zumbusch). Identical gaps were found easily, but less frequently, in the normal-appearing skin of psoriatic patients, and only rarely in the normal-appearing skin of controls. In these initial studies, multiple sections, but not true serial sections, suggested that the gaps were confined to the intercellular junctions where 2 or more endothelial cells met. The gaps were indistinguishable by electron microscopy (EM) from those produced by the intradermal injection of histamine. Reconstruction of the gaps from serial sections at that time was not technically feasible. Following the design and implementation of a computer graphics system for the reconstruction of serial EM and light microscopic sections in 3 dimensions [3], we reexamined the problem of gap formation to determine the mechanism(s) involved and to see whether the gaps were distinctive enough to be used as morphologic markers of psoriasis.

MATERIALS AND METHODS

We reexamined the tissue blocks from 3 patients with psoriasis (2 plaque, 1 pustular psoriasis) who had been shown to have endothelial cell gaps [2]. We also reexamined specimens from 2 normal individuals who had developed endothelial cell gaps 5 min after the intradermal injection of histamine into flexor forearm skin. The psoriatic group consisted of 2 men (54 and 59 years) and 1 woman (45 years). The histamine group consisted of 2 women (50 and 52 years). Each specimen was serially cut at approximately 80–90 nm for 120–140 sections, collected on single-slot Formvar film-coated grids and stained with uranyl acetate and lead citrate before viewing. The 50th section was searched for gaps and if found, the same gaps were looked for in sections 49 to 1 and 51 to 140 and photographed. In this way the entire span of a gap was examined. If a gap was not found in the 50th section, a survey was made forward and backward from this spot to find gaps and when found, the same gap was photographed forward and backward over its full extent. Only gaps that were not associated with inflammatory cells were studied. A total of 57 psoriatic gap formations were photographically enlarged, outlined on the print with a portion of the endothelial cell(s) on either side, traced onto transparent acetate sheets, aligned with fiducial marks made with reference to tissue landmarks, and entered into the computer by tracing the outlines on a digitizing pad. The X, Y coordinates of these outlines were processed by the computer and the gaps were then reconstructed in 3-D from various viewing angles. The details of the computer system are described in another paper [3]. All 57 gaps were studied in the same way.

Forty-one histamine gaps were reconstructed in an identical fashion. In addition, we had available for review 120–140 serial EM micrographs of vessels in buttock skin from 2 healthy non-psoriatic young female volunteers (28 and 30 years), 1 healthy aged man (80 years), and 1 nonpsoriatic juvenile diabetic woman (32 years). All of the vessels studied were in cross section.

The histamine-induced gaps were produced in the following manner as described previously [4]. Histamine phosphate (Eli Lilly and Co., Indianapolis, Indiana) was injected intradermally into the normal-appearing flexor forearm of volunteers. The amount injected was either 0.05 ml of a 1 mg/ml or 0.1 mg/ml concentration of the commercially available preparation. No difference in effect was noted between the 2 concentrations. The resulting wheal was outlined in its greatest diameter and biopsied either in the center or along the border opposite the site of injection. Since the wheals measured 2–3 cm in diameter, the 3- and 4-mm punch biopsies which were taken 5 min after the injection of the histamine phosphate did not include the injection sites. As controls, 0.05 ml of physiologic saline was injected intradermally at another site on the flexor forearm. All biopsies were performed 5 min ± 1 min after the injection of histamine or saline. All skin biopsies including those of psoriatic plaques were performed after a ring of anesthesia with 1% lidocaine without epinephrine had been placed around the sites to be injected with histamine or saline or biopsied. The rings of anesthesia were never contiguous with the outlines of the histamine-induced wheals, the sites of saline injection, or the perimeter of the psoriatic plaques.
These studies were approved by the Human Investigations Committee at Yale.

RESULTS

The detailed survey of 120–140 serial sections in each of the 4 control patients failed to disclose any gap formations.

As in our earlier studies, gaps were found mostly in the postcapillary venules of the horizontal plexus, and less frequently in the venous capillary loops of psoriatic lesions. The endothelial cell gaps had one of 3 configurations: (1) a separation between the cells with fragments of one or more endothelial cells still attached to one another by intercellular junctions at one edge of the gap; (2) a separation between 2 cells with several islands of cytoplasmic fragments within the gap (Fig 1); (3) a separation between 2 endothelial cells without any intervening islands of cytoplasm. There were various sized non-membrane-bound vacuoles within these cytoplasmic islands, as well as in the endothelial cells at the edges of the gap (Fig 2). There was no evidence of cell death or fixation artifact in the tissues. Also there were no indentations of nuclear membranes nor did the nuclei bulge into the lumens of the vessels implying endothelial cell contraction.

Computer reconstructions indicated that although gaps were present at intercellular junctions they were also seen as isolated holes within the cells themselves. Of the 57 psoriatic gaps reconstructed, 27 were present only at intracellular sites (Fig 3), 28 were found at noncontiguous intracellular and intercellular sites simultaneously (Fig 4), and 2 were located exclusively at intercellular junctions. The gaps were linear or oval in shape, extended parallel to the long axis of the vessels, and ranged in width from 0.03–2.0 μm and from 0.05–7 μm in length. Fig 4 is a reconstruction of a representative gap. The 2 endothelial cells are not cleanly separated. There is bridging of cytoplasm across the gap where the cells are still adherent. There is a finger-like process of cytoplasm jutting into the gap as well as an isolated hole within one of the cells. A section passing through this isolated hole and

Figure 1. Gap formation in psoriatic vessel. Endothelial cell E2 forms a gap with E1 which is the source of the islands of cytoplasmic processes in the gap. Pericyte (P) also contributes a cytoplasmic process to gap. R = erythrocyte. Bar = 1 μm.

Figure 2. Gap formation in psoriatic vessel. Arrows indicate non-membrane-limited vacuoles in endothelial cells (E) at edge of gap. P = portion of pericyte. Bar = 1 μm.

Figure 3. Computer reconstruction of intracellular endothelial cell gap in psoriatic vessel. Only the portion of the endothelial cell which contains the gap was reconstructed. Gap viewed from abluminal surface. Arrow indicates longitudinal axis of vessel. Z = 180, X = 180, Y = 60.
continuing across the gap to include the cytoplasmic process jutting into the space would produce cytoplasmic islands similar to those shown in Fig 1.

Pericytes normally have close multiple appositions with the endothelial cells of the cutaneous microvessels as illustrated in Fig 5. This close proximity of pericyte to endothelial cell was frequently associated with a pericytic cytoplasmic process sticking into the gap itself (Fig 5). A reconstruction of this phenomenon is illustrated in Fig 6.

We studied 41 histamine-induced gaps by the same techniques. The gaps were detected 5 min ± 1 min after the intradermal injection of histamine. The positions of the gaps were similar to those found in psoriatic lesions. Thirty-seven were present at both intercellular and intracellular sites and 4 were present only at intercellular junctions. There was none seen exclusively at intracellular sites. Bulging and indentations of endothelial cell nuclei were not found. Gaps were not observed at the sites of saline injection.

**DISCUSSION**

The endothelial cell gaps found in psoriatic vessels and those produced by intradermal injections of histamine appear to be morphologically identical. We have found endothelial cell gaps only rarely in normal nonpsoriatic skin [2]. The inability to detect gaps in 4 additional specimens from nonpsoriatic subjects that were examined in 120–140 serial sections is further evidence for the distinctiveness of this vascular abnormality. Although gaps may not be diagnostic or unique to psoriasis, they may still be distinctive enough to serve as a morphologic marker to screen for latent psoriatics, since they are easily found in the normal-appearing skin of psoriatic patients [1,2].

The mechanism of gap formation is still not completely understood. Endothelial cell contractility had been proposed as a mechanism for histamine-induced gap formation. Majno et al [5] had proposed that endothelial cell contraction, produced by the direct action of histamine was responsible for the intercellular gaps. As evidence for contraction they cited the association of indented, wrinkled, and "pinched" nuclei with the presence of these gaps.

**Figure 4.** Computer reconstruction of endothelial cell gaps shown in Fig 1. Gap is present at intercellular junction but there are a few points (arrowheads) where the cells are still adherent and form small bridges. The darker cell extends a cytoplasmic process into the space and also contains another gap that is not continuous with the intercellular one. Only the portions of the endothelial cells containing the gaps were reconstructed. Sections rotated 60° on Y axis. Gaps being viewed from luminal surface. Arrow indicates longitudinal axis of vessel. Bar = 1 μm.

**Figure 5.** Relationship of pericyte to gap. Pericyte makes contact with endothelial cell (arrow) and inserts a cytoplasmic process into the gap itself (asterisk). Bar = 0.08 μm.
They excluded dilatation of the postcapillary venules with increased flow as being a factor because gaps still formed in the absence of vascular congestion. Hammersen [6] recently reviewed this controversial issue. Neither we nor Hammersen have observed indented or “pinched” nuclei in association with histamine gaps. Hammersen also found irregularly outlined nuclei in the absence of gaps [6], Hulstrøm and Svensjø [7], who studied bradykinin-induced endothelial gaps in the hamster cheek pouch by intravital fluorescence microscopy in correlation with EM, also failed to find “pinched” or folded endothelial cell nuclei. In addition, in our studies of histamine-induced gaps, the postcapillary venules were markedly dilated with an extremely thin endothelium. We did not observe nuclear deformations or endothelial cell nuclei bulging into the lumen as one might expect if the endothelial cells had contracted. Although endothelial cells contain filamentous structures that can be identified as actin immunohistochemically, this is insufficient evidence to establish that they perform a contractile function [4].

Svensjø, Afors, and Rutli [8,9] studied histamine- and bradykinin-induced endothelial cell gap formation in the hamster cheek pouch. Their studies indicated that histamine produced leakage in postcapillary venules while simultaneously causing arteriolar vasoconstriction. In another study they were able to block the permeability-increasing properties of bradykinin on the postcapillary venules with the β2 stimulator, terbutaline, without decreasing vascular blood flow [9]. These experiments suggested that the permeability effect of bradykinin, and by inference histamine, produce their effects by direct competitive action on the endothelial cells at the postcapillary venular level independent of arteriolar construction or vasodilation. However it should be noted that the effect of histamine on microvessels varies among species. It produces marked arteriolar vasoconstriction of arterioles in rodents, slight arteriolar constriction in cats, and arteriolar vasodilatation in dogs, monkeys, and humans [10].

Hammersen [6] proposed another explanation for gap formation that was suggested by studies of various types of experimental and human edemas. He observed an elaboration of cytoplasmic processes along the luminal and abluminal surfaces of endothelial cells. These projections also occurred at the endothelial interfaces, producing an incomplete fitting of the apposing cell membranes. He proposed this as the first step in the formation of an endothelial gap as the adjoining cells were gradually pushed apart by the increasing amount of plasma fluid leaking out into the interstitial space.

All investigators who have studied histamine gaps have localized them to intercellular junctions. We have shown that they also occur within the cell. In psoriasis, the gaps were more common within the cell than at intercellular junctions. It is very difficult to demonstrate the precise location of gaps by routine EM sections alone. Fox, Galey, and Wayland [11] also studied histamine gap formation in mesenteric microvasculature by computer reconstruction. They published photographs of 3 gaps all of which were at interendothelial cell junctions. Their reconstructions also showed that what appeared to be isolated cytoplasmic fragments of endothelial cells in the gaps, as visualized by routine EM, were actually portions of adjacent endothelial cells forming bridges of cytoplasm across the gap. Figs 1 and 4 in our paper show similar phenomena. Fox et al [11] proposed that the irregular oval holes with cytoplasmic bridging in their 3 examples could not be due to simple endothelial cell contraction. The geometry of the holes suggested to them that there might be a local weakness in the “intercellular cement” at these regions; there might be a local attachment of contractile fibers to the endothelial membrane at these junctions; or both conditions might be present to produce irregular holes when the endothelial cell contracted [11].

Our studies emphasize that the gaps are linear to oval, occur within the cell in the zone between the nucleus and intercellular junction as well as at the junction, and can be irregular in outline because of cytoplasmic processes that project into the gaps. The appearance of the gaps suggests that they may have been formed by the tearing of cytoplasm, implying that cellular injury rather than a physiologic mechanism is responsible for their formation. The orientation of the gaps, which is parallel to the long axis of the vessel, is also compatible with the tearing of cytoplasm as might be produced by the stretching of endothelial cells resulting in areas of attenuation leading to rips. The intercellular junctions of the postcapillary venules have been shown by Simionescu et al [12] to be the loosest of any in the microvasculature bed.

The psoriatic gaps might be explained in a similar way. The capillary loops and postcapillary venules in psoriasis are markedly dilated and the blood flow is greatly increased in psoriatic plaques. There are increased numbers of mast cells in psoriatic lesions which might play a role in facilitating the increased flow and the development of gaps [13]. The vessels in psoriatic plaques are chronically dilated and they may remain so for up to 5 months after the skin has returned to a normal appearance following therapy [14]. In addition, gaps are easily found in the normal-appearing skin of psoriatic patients.

The experiments of Majno et al [5] and Svensjø et al [8,9] strongly suggest that increased blood flow with vascular congestion does not play a role in gap formation in their systems. These workers provide strong evidence for the direct action of histamine on the endothelial cell to produce gaps. They propose that gap formation is produced by endothelial cell contraction. However, Hammersen [6] has made a strong case against endothelial cell contractility. The reconstructions by Fox et al [11] and ourselves clearly show that gap formation may be a pathologic, nonphysiologic event because of its morphology. Another hypothesis to be tested is whether histamine could damage the plasma membrane of the endothelial cell or produce intracellular edema that would result in pathologic gap formation. The vascular changes in the endothelial cells shown in Fig 2 could be either the cause or the effect of the gap formation. Unfortunately physiologic investigations dealing with the induction of increased vascular permeability by histamine or bradykinin have not yet been correlated with ultrastructural studies of the sequential steps in gap formation.

Gaps are probably formed by more than one mechanism. The species involved, the site and type of microvascular bed, and the concentrations of mediators will need to be considered in studying gap formation. The role of endothelial cell contractility in the
formation of gaps also needs to be reevaluated because of the recent work by Joyce et al [15, 16] indicating that pericytes, which have multiple close appositions of the tips of their processes to the underlying endothelium, contain proteins essential for contraction in higher concentration than any other cells associated with the microvasculature, except for smooth muscle cells.

In normal skin, histamine-induced gaps are easily found 5 min after intradermal injection, but are rarely observed 3-4 h later (Braverman, Keh-Yen, unpublished data). In psoriasis, gaps persist after successful therapy of lesions and are easily found in the normal-appearing skin of psoriatics [2]. Yet, by reconstruction, histamine-induced gaps are morphologically identical to those found in psoriasis. It remains to be determined whether the factors responsible for gap formation following the injection of histamine and the "spontaneously" occurring gaps in psoriasis are also identical.

REFERENCES