

REJECTION OF FIRST-SET SKIN ALLOGRAFTS IN MAN

The Microvasculature Is the Critical Target of the Immune Response*

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Although it is agreed that mononuclear cells, especially T lymphocytes, have an essential role in the rejection of vascularized grafts in previously unsensitized, genetically incompatible hosts (i.e., first-set set allografts) (1-10), it is not yet clear how inflammatory cells or their products actually effect graft destruction. One popular view, originating with the classic studies of Medawar (1) and supported by many subsequent authors (2, 7, 11), holds that the epithelial elements of the graft bear the brunt of the immune response and are destroyed by an invasive-destructive infiltration of inflammatory cells. Such a concept is supported by in vitro studies demonstrating that lymphocytes, macrophages, and perhaps, granulocytes are able to destroy individual epithelial target cells either by direct cell contact or by secretion of cytotoxic mediators (12-18).

An alternate possibility suggests that the host's immune response is directed at least in part against the blood vessels of the graft which are thought to share histocompatibility antigens with epithelial cells (4, 6, 10, 19-22). According to this view, allografts are rejected as a direct consequence of vascular occlusion and tissue ischemia. This hypothesis is supported by the observation of intimal thickening and lymphocytic infiltration of major graft vessels in the course of renal (21), cardiac (23), and pulmonary (24) transplant rejection and by recent histochemical evidence describing loss of ATPase activity in the vessels of rejecting renal allografts (25). However, other morphologic evidence of vascular injury in cell-mediated allograft rejection is less secure. Some authors have described widespread vascular thrombosis in skin-allograft rejection (19, 22) and from this have inferred a preceding endothelial lesion, but most investigators regard vascular thrombosis as a late and sporadic event in the rejection process. In addition to infiltration of the epithelium by mononuclear cells, Waksman (4) reported damage to both arteries and veins as well as "mononuclear cell thrombosis" in rat skin allograft rejection. However, these studies taxed the limits of conventional light microscopy and were not supported by subsequent electron microscope studies in the rabbit (11). In view of these contradictions, a predominantly vascular mechanism for the cell-mediated rejection of first-set allografts has not gained wide acceptance.

Having recently demonstrated microvascular lesions in delayed hypersensitivity

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reactions (26, 27), we were prompted to reexamine the pathogenesis of first-set skin allograft rejection using morphologic techniques that permitted both extensive vessel sampling and unequivocal identification and evaluation of vessel endothelial cells. We here report that widespread microvascular damage is indeed a characteristic and early feature of the cellular immune response to first-set human skin allografts and is qualitatively similar to, but substantially more intense than, that occurring in delayed hypersensitivity reactions (26, 27). Microvascular damage invariably preceded evidence of significant epithelial necrosis, and affected initially and primarily those venules, arterioles, and small veins enveloped by lymphocytes and other inflammatory cells. These findings strongly suggest that endothelial cells of the microvasculature are the critical target of the immune response in first-set skin allograft rejection in man, and that rejection itself can be attributed largely to ischemic infarction resulting from extensive microvascular damage.

Materials and Methods

Skin Grafting Procedure. The design and conduct of these experiments were approved by the Human Studies Committee of the Massachusetts General Hospital. Volunteers were accepted only after the results of a thorough medical history, physical examination, and routine laboratory tests proved to be within normal limits.

Split-thickness thigh skin ($\cong 0.3$ -mm-thick) for use as allografts was removed with a dermatome from one of the investigators and from another adult male volunteer, packed in sterile, saline-soaked gauze, and sutured in place on prepared sites ($\cong 2.5 \times 5$ cm) within 3 h of removal. Each donor provided allografts for four recipients. Split-thickness graft beds were prepared by free-hand dissection on both deltoid surfaces of eight adult male volunteers under local anesthesia. Allografts were transplanted to both arms of each recipient. All grafts were covered with compression bandages except at times of examination and biopsy.

Ideally, skin would also have been removed from each recipient with a dermatome for reimplantation as an autograft. Because this was impractical, four recipients received as autografts the skin removed during preparation of a second graft bed on the right arm. However, free-hand dissected autografts were approximately three times thicker than dermatome-prepared allografts, and we were concerned that these thicker grafts might experience difficulty acquiring a blood supply. For this reason, autografts for the first four recipients were freed from the graft bed beneath and on three sides but the fourth side (2.5-cm. dimension) was allowed to remain in continuity, thereby creating a skin flap which was sutured in place.

Graft Biopsy, Tissue Processing and Human-Leukocyte-Antigen (HLA)¹ Typing. Grafts were examined and biopsied on day 3 or 4 and daily thereafter until day 12-13. Biopsies were taken with a 4-mm punch using 2% xylocaine without epinephrine. Tissue was fixed in paraformaldehyde-glutaraldehyde for 5 h at room temperature and was processed for giant, 1- μ m-thick Epon (Shell Chemical Co., New York) sections and for electron microscopy as previously described (26, 28). A total of 126 biopsies from allografts and 42 from autografts was studied in giant, 1- μ m-thick Epon sections. One-half of these were additionally studied by electron microscopy and the other one-half by immunofluorescence. For the latter procedure, cryostat sections of fresh, frozen tissue were stained with specific fluoresceinated goat or rabbit antisera to human fibrinogen/fibrin, polyvalent human gamma globulin, C'3, and human albumin, and examined in a Zeiss fluorescence microscope (Carl Zeiss, Inc., New York) as previously described (26).

HLA typing and tests for development of anti-HLA antibodies in recipients were performed using published procedures (29, 30).

Results

At least three HLA incompatibilities distinguished each recipient from his donor

¹ Abbreviation used in this paper: HLA, human leukocyte antigen.

TABLE I
Blood Group and HLA Serotyping of Skin Allograft Donors and Recipients

Donors				Recipients				Day of rejection (gross)	Number of HLA-A and-B locus incompatibilities between donor/recipient
Number	Age	ABO-Rh ₀ D	HLA phenotype	Number	Age	ABO-RH ₀ D	HLA phenotype		
1	35	0+	A3, 10; B8, x*	1	34	A+	A2, 9; B7, 27	10-11	4
				2	32	B+	A9, x; * Bw35, 40	10	4
				3	38	0+	A3, 29; B7, 12	11-12	3
				4	39	0+	A1, 2; B40, —	11	3-4
2	38	0+	A3, 29; B7, 12	5	55	B+	A1, 3; B17, —	12	3
				6	22	B+	A2, 9; B13, w35	11-12	4
				7	34	0+	A1, 2; B8, —	11-12	4
				8	34	0+	A1, 2; B8, 12	11	3

* x, undefined HLA alloantigen.

(Table I). All recipients had a negative cross match against donor lymphocytes at the time of grafting. Moreover, recipients lacked detectable cytotoxic antibodies against donor lymphocytes at 7 and 15 d after transplant.

After initial revascularization, all 16 allografts were sloughed between days 10 and 12. By contrast, all autografts healed in place and survived indefinitely.

Microscopic Appearance of Allografts

EARLY HEALING AND LEUKOCYTE INFILTRATION. Healing of allografts proceeded in accord with published descriptions (1-3, 7, 10, 11, 19). In brief, blood flow was established by 3-4 d as judged both by the clinical appearance of the grafts and by the presence of erythrocytes and plasma within small vessels at all levels of the dermis. Nonetheless, the junction between the graft (tissue of donor origin) and the graft bed (tissue of recipient origin) could be positively and precisely identified through the time of graft rejection. This interface was marked by (a) trace residual deposits of matted fibrin, erythrocytes, and other debris dating from the time of surgery (Fig. 1) or (b) by proliferating epithelium, derived from transected hair follicles, in the plane of the graft-graft bed interface (Fig. 2A). As early as day 5, allografts could be differentiated microscopically from autografts by the appearance of a mononuclear cell infiltrate about nearly all venules and adjacent arterioles of deeper portions of the allograft and the subjacent graft bed (Fig. 1, levels 2-4; Fig. 2A, B). Accumulating cells consisted largely (>90%) of small lymphocytes and lymphoblasts, the majority of which remained in close proximity to the venules from which they emigrated, forming the perivenular cuffs characteristic of delayed hypersensitivity reactions (2, 7, 26). Monocytes and macrophages were represented in these perivenular infiltrates in small numbers, and plasma cells were observed only rarely.

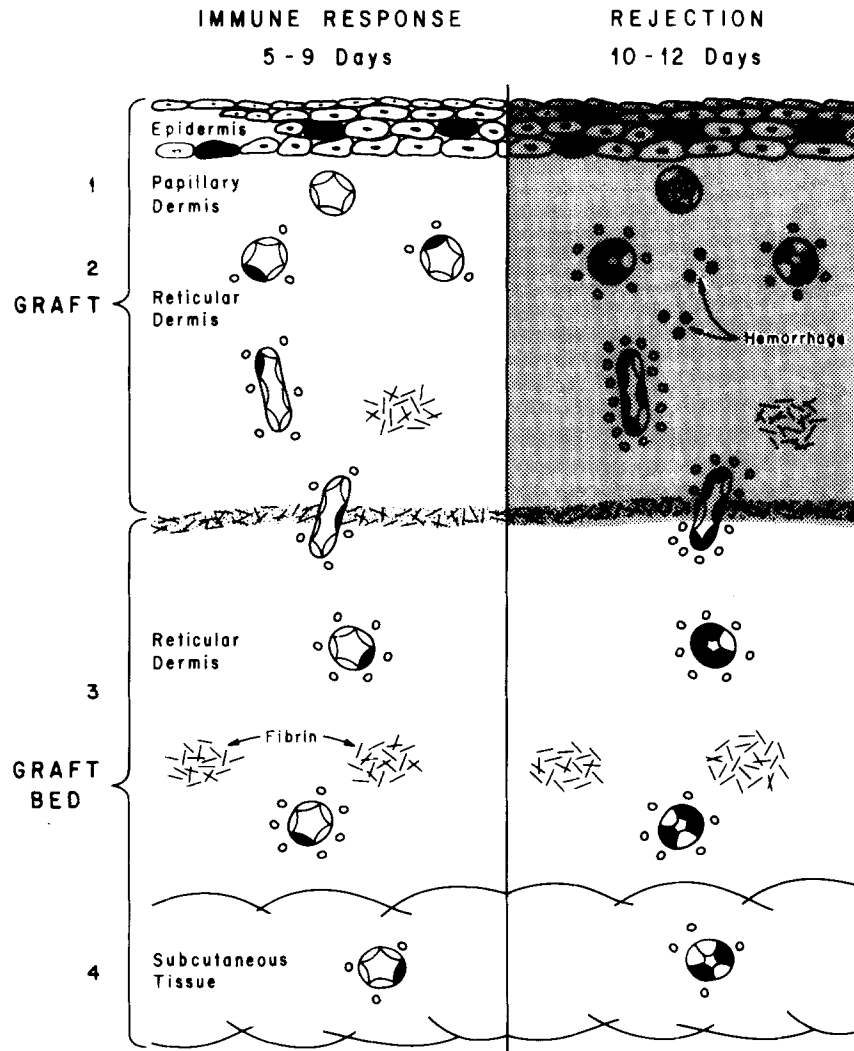
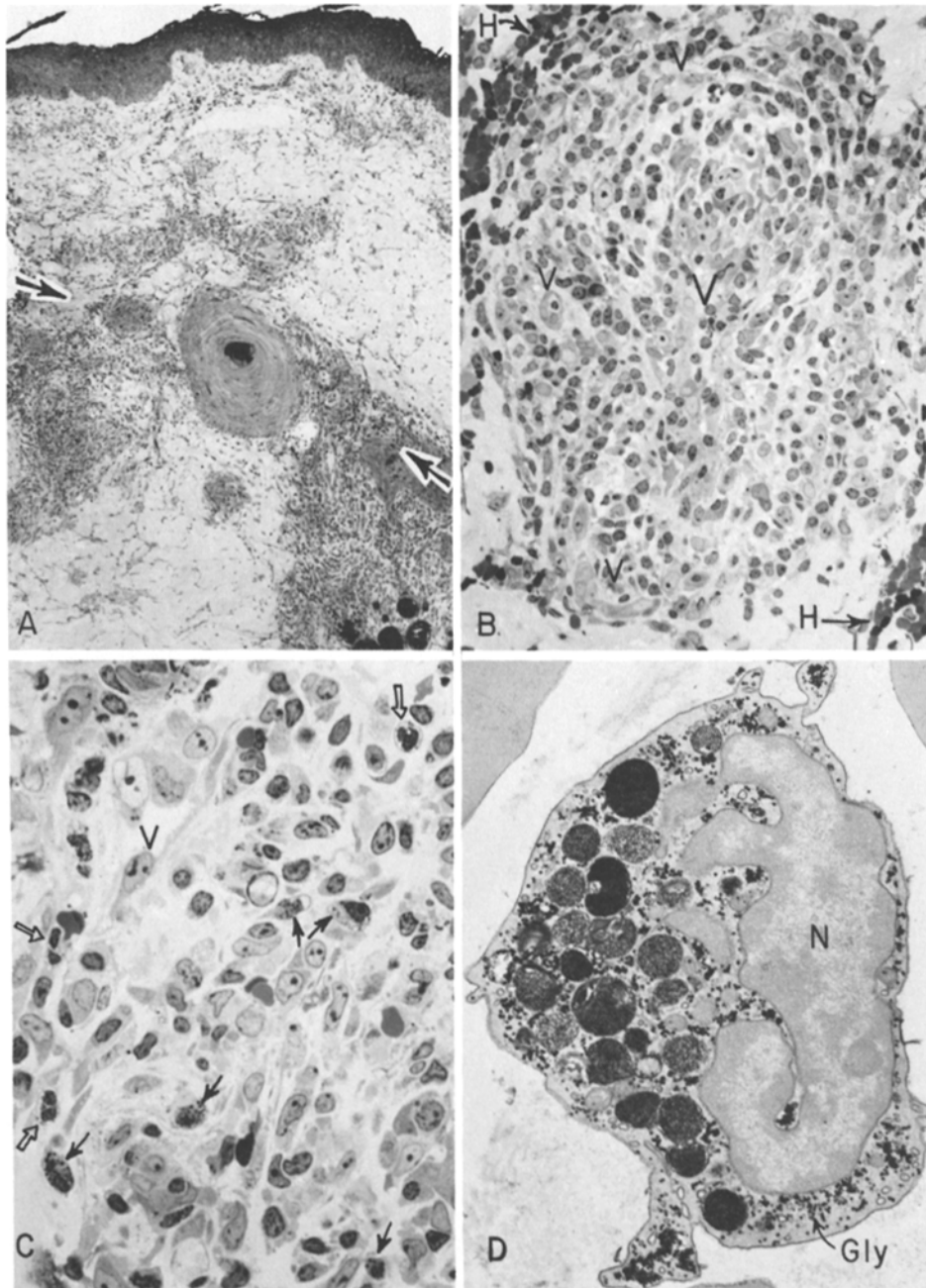


FIG. 1. Schematic diagram of skin allograft rejection. The graft and graft bed are separated by a thin layer of matted fibrin and debris which is bridged by blood vessels. Graft blood vessels of the papillary dermis (level 1) are not enveloped by cuffs of lymphocytes, whereas deeper graft vessels (level 2), as well as vessels of the graft bed (levels 3 and 4), are so circumscribed. Inflammatory cells, mostly lymphocytes, are indicated by open circles and are concentrated about blood vessels. Damaged endothelial cells are colored black. Dyskeratotic foci of epidermis and accompanying inflammatory cells (not separately indicated) are also indicated in black. Infarction of the graft after day 10 is designated by cross-hatching.

A finding described in guinea pig allograft rejection (31), but one not previously appreciated in human skin grafts, is that basophilic leukocytes also participated prominently in the cellular infiltrate, appearing initially in the same perivascular distribution as lymphocytes, but several days later (Fig. 2 C, D). In contrast to lymphocytes, the majority of basophils did not persist about venules but rather became disseminated through the graft dermis and occasionally infiltrated the epi-

dermis as well. Basophils became increasingly numerous in allografts a day or two before rejection, accounting for up to 5% of infiltrating cells. Basophils underwent a form of piecemeal degranulation (Fig. 2C) as previously described in contact allergy (32). Eosinophils were also sometimes present in small numbers (33) but they appeared sporadically and only after the infiltration of basophils.



For the most part, graft epithelial elements appeared normal and were indistinguishable from their counterparts in autografts through day 9 or 10. However, the epidermis and especially the hair follicles did exhibit scattered foci of dyskeratosis (individual cell necrosis) and intercellular edema accompanied by a localized cellular infiltrate composed mostly of lymphocytes but also including neutrophils and occasionally basophils. Such areas of dyskeratosis never involved >5–10% of the epithelium.

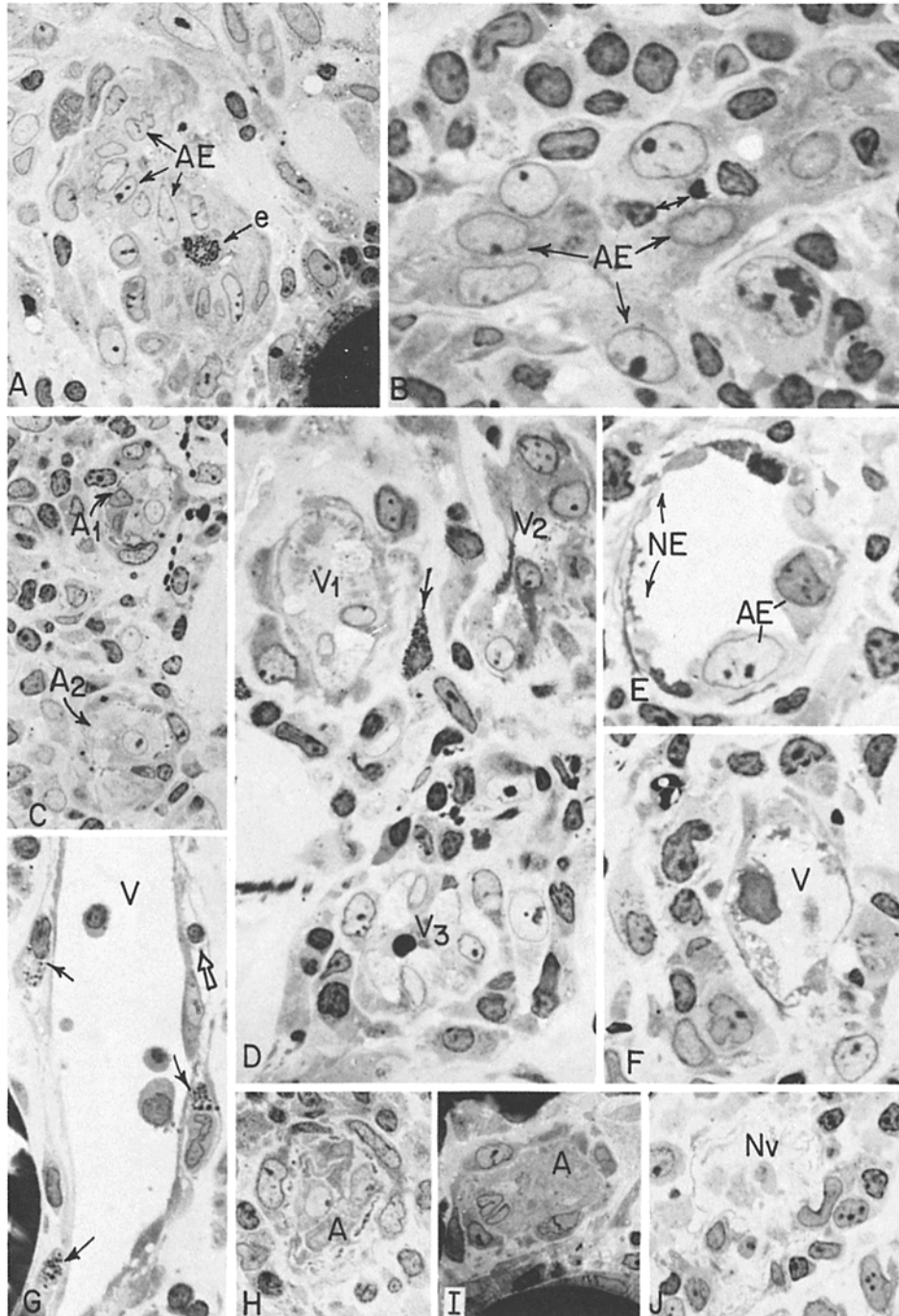
MICROVASCULAR ALTERATIONS ACCOMPANYING CELLULAR INFILTRATE. The microvasculature of both the allograft and the graft bed exhibited identical and striking alterations which were particularly evident in those venules and arterioles enveloped by cuffs of lymphocytes (Fig. 1, levels 2–4; Figs. 2A–C, 3, 4). As in contact allergy (26, 27), pericytes and endothelial cells at all levels appeared activated (hypertrophied) and frequently exhibited mitoses; hypertrophied endothelial cells sometimes bulged into, and apparently compromised, vascular lumens (Figs. 2B, C; 3A–E, H, I; 4). However, the most striking new finding was the concomitant development of widespread and progressive endothelial cell injury (Figs. 3C–F, I, J; 4). Microscopic and ultrastructural evidence of injury was evident shortly after the appearance of perivascular lymphocyte cuffs (days 5–6) and included endothelial cell swelling and cytoplasmic lucency; membrane swelling, blebbing, and disruption; nuclear pyknosis; and focal sloughing of entire endothelial cells, permitting exposure of circulating elements to the vascular basement membrane. Endothelial cell damage was often accompanied by edematous thickening of the basement membrane zone (V₁, Fig. 3D).

In contrast to contact dermatitis, where analogous microvascular injury was relatively slight and self-limited (26, 27), endothelial cell damage in allografts was well developed by day 6 and became increasingly prominent with the passage of time and progression of the cellular infiltrate (Table II). By day 10, the vast majority of vessel profiles counted exhibited necrosis of at least some endothelial cells. Both vessels of the graft and recipient graft bed were affected but level-2 and -3 vessels were generally damaged earlier and more extensively than those in levels 1 and 4. In addition, many vessel lumens were dramatically narrowed or even occluded by encroachment of activated, or damaged and swollen, endothelial cells. In some instances, vessels lost their normal organization altogether and appeared as clusters of viable or damaged, but still identifiable (by electron microscopy), endothelial cells without relation to any recognizable lumen. Larger arteries and veins (Diam >50 μ m) of the graft bed were generally less severely injured but were occasionally infiltrated with lymphocytes or basophils (Fig. 3G). By day 10, thrombosis developed in some graft vessels (Fig.

FIG. 2. A. 8-d allograft exhibiting extensive perivascular inflammatory cell infiltration at level 2 of graft and levels 3 and 4 of graft bed. Arrows indicate the graft-graft bed junction which is marked by trace fibrin deposits (not visible at this low magnification) and by a hair follicle. Proliferation of hair follicle epithelium in the graft-graft bed junction was commonly observed in allografts, but not in autografts. There is focal hemorrhage but little lymphocyte infiltration in the superficial graft dermis and the epidermis appears intact. B. Perivascular infiltrate, nearly all lymphocytes and lymphoblasts, surrounding level-2 allograft vessels. Note hypertrophied endothelial cells of vessels (V) and interstitial hemorrhage (H). C. Dermis of graft bed reveals a cellular infiltrate rich in basophils (arrows), some of which exhibit nearly complete degranulation (open arrows). Venule (V) shows striking endothelial cell activation. D. Electron micrograph of infiltrating dermal basophil with generally good particle content in cytoplasmic granules. N, nucleus; Gly, glycogen. A–C, 1- μ m-thick, Giemsa-stained Epon sections. A, \times 50; B, \times 315; C, \times 650. D, electron micrograph, \times 15,000.

5 B) as has been described by others (1, 19), but the lumens of graft-bed vessels generally remained free of fibrin clots despite extensive endothelial cell injury.

The physiologic significance of these vessel changes was manifest as early as day 5 by the leakage of plasma from vessels, leading to tissue edema and fibrin deposition



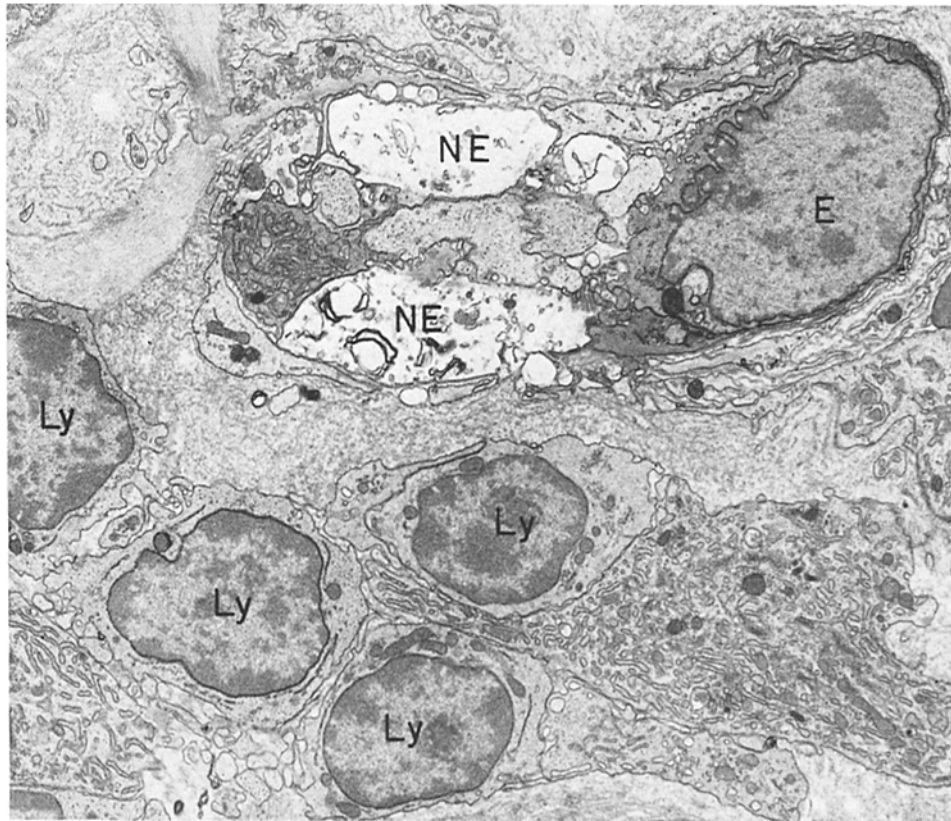


FIG. 4. Electron micrograph of a typical venule from an 8-d allograft exhibiting both endothelial cell activation (E) and necrosis (NE). Vessel is cuffed by small lymphocytes (Ly). $\times 6,500$.

that was appreciated microscopically and clinically as swelling and induration (26, 34). After day 7, more extensive vessel damage permitted the progressive extravasation of erythrocytes (Figs. 2A, B); in advantageous sections, sites of extravasation could be localized to areas of endothelial damage. Extensive hemorrhage into the graft dermis was common by day 9 or 10 (Fig. 5A). These changes, which are consistent with marked diminution or cessation of local blood flow, generally preceded widespread necrosis of graft epithelial elements by 24–48 h.

GRAFT REJECTION. The final phase of the graft rejection process consisted of the relatively sudden (within 1–2 d) necrosis of scattered islands of graft tissue; these islands initially measured up to 1 cm in largest diameter but coalesced over 24–48 h to involve most of the graft. Microscopically, such areas exhibited coagulation necrosis

FIG. 3. Microvascular endothelial cell activation and necrosis in allografts undergoing immunologic rejection. Activated endothelium (AE) was identified in vessels of both the graft (B) and the graft bed (A, E). Vascular lumens were sometimes extensively compromised by activated endothelium, seen compressing an intraluminal eosinophil (e) in A and several lymphocytes (double-headed arrow) in B. The endothelium of both venules (V) and arterioles (A) of the graft (C) and graft bed (D–F, I, J), exhibited injury as indicated by swollen, lucent ghost-like cell remnants (V₁ and V₃, D) or by ragged loss of endothelium (V₂ in D; NE in E, F). Altogether necrotic vessels were commonly observed at later intervals (Nv, J). The walls of larger venules and veins were not uncommonly infiltrated by basophils (arrows) or lymphocytes (open arrow) as in G. All 1- μ m-thick, Giemsa-stained Epon sections. A, C, G–J, $\times 645$; B, E, F, $\times 1,000$; D, $\times 790$.

TABLE II
Quantitative Evidence of Progressive Microvascular Injury Preceding First-Set Skin Allograft Rejection

Vessel level	Number of vessel profiles counted						Percentage of vessel profiles exhibiting necrotic endothelium					
	Allografts			Autografts			Allografts			Autografts		
	Day 6	Day 8	Day 10	Day 6	Day 8	Day 10	Day 6	Day 8	Day 10	Day 6	Day 8	Day 10
1 Papillary dermis, graft	146	158	150	—	28	37	30.8	42.4	76.6	—	0	2.7
2 Deep dermis, graft	198	307	216	—	41	87	41.4	59.6	82.4	—	2.4	2.3
3 Dermis, graft bed	89	197	277	—	31	62	45.0	65.5	79.8	—	3.2	0
4 Subcutis, graft bed	41	99	98	—	7	67	34.1	40.4	45.9	—	0	0
Total	474	761	741		107	253						

Biopsies of 6-, 8-, and 10-d allografts from all eight recipients were studied in 1- μ m Epon sections. This technique permitted positive vessel identification and evaluation. All vessels encountered at the four levels defined in Fig. 1 were scored for the presence of damaged endothelium. Data are expressed as the total numbers of vessel profiles encountered and the percentage of these that exhibited one or more necrotic endothelial cell. 8- and 10-d autografts from three recipients were similarly assessed. Chi-square analysis revealed that in every instance (days 8 and 10, vessel levels 1-4) allograft vessels exhibited significantly more endothelial necrosis than corresponding autografts ($P < 0.005$).

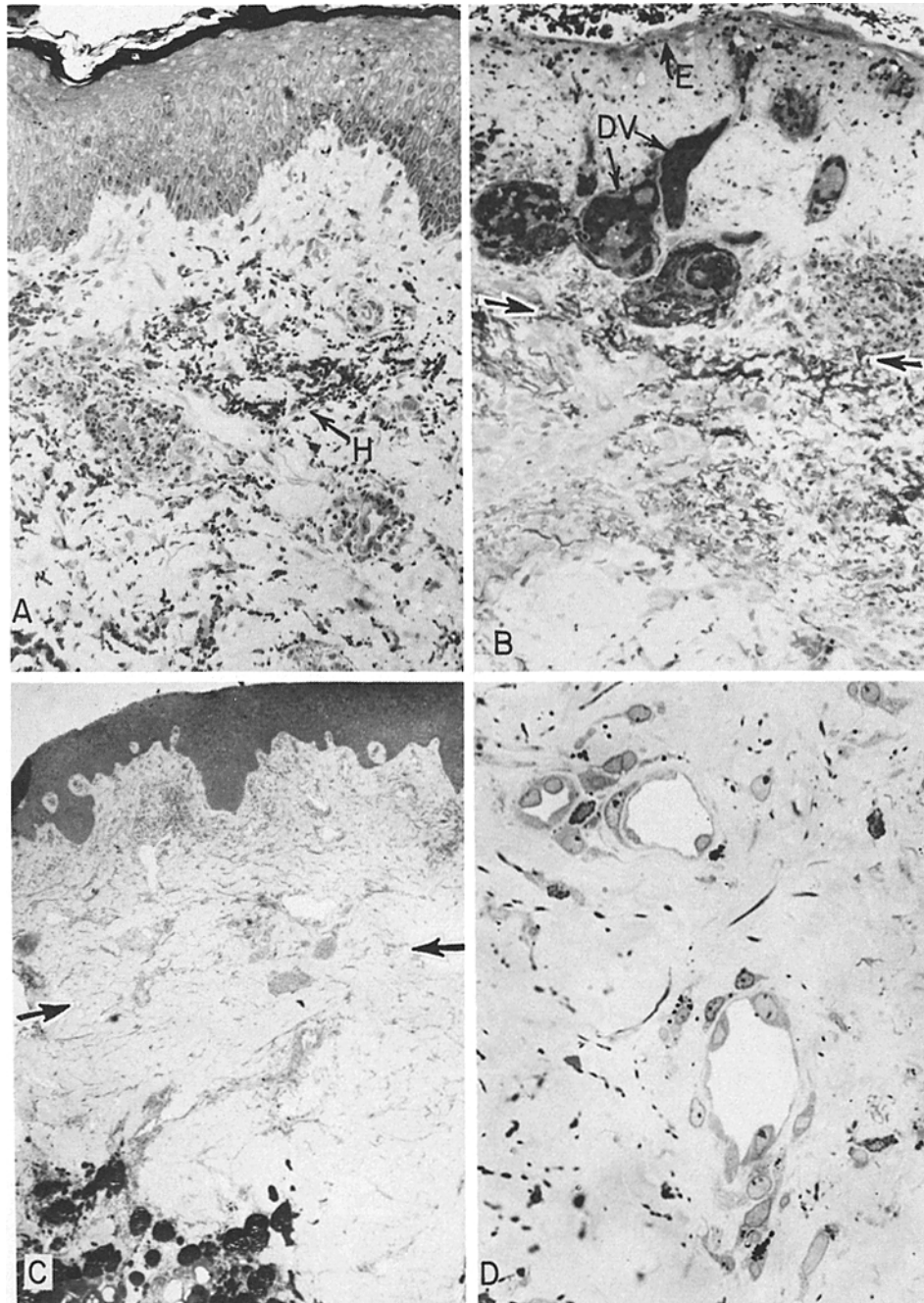
of the entire thickness of the graft including epidermis, appendages, vessels, and inflammatory cells (Fig. 5 B), and thus resembled tissue undergoing ischemic infarction in other clinical settings following cessation of local blood flow (e.g., myocardial infarction). While necrosis was largely confined to the graft itself, contiguous portions of the superficial graft bed (tissue of recipient origin) were sometimes also damaged (Fig. 5 B).

The relatively sudden rejection episode could not be explained by individual, persistent anatomic contacts between graft-epithelial elements and any type of inflammatory cell. Damage to epithelial cells in dyskeratotic foci of the epidermis and appendages was associated with infiltrating lymphocytes and was evident as early as day 5. However, this process progressed slowly, and even at the time of rejection involved <5-10% of the epithelium.

Microscopic Appearance of Autografts. Healing of autografts resembled that of allografts until approximately day 5. Thereafter, the vessel activation that accompanied revascularization of autografts ceased and perivascular mononuclear cell infiltrates and associated vascular endothelial cell necrosis never appeared in either the graft or the graft bed. Free autografts differed from skin flap autografts only in that they were slower to acquire a vascular supply and developed transient early foci of epithelial injury. By days 10-11, all autografts were well healed with a patent microvasculature and healthy-appearing epidermis and appendages (Fig. 5 C, D).

FIG. 5. A. 9-d allograft exhibiting perivascular lymphocytic infiltrate, interstitial hemorrhage (H), but an as-yet intact epidermis. B. 11-d allograft exhibiting characteristic pattern of infarction. The now desiccated graft has lost more than one-half of its vertical dimension (compare with A, photographed at nearly the same magnification) and is characterized by a necrotic epidermis (E) and prominent, dilated, and thrombosed blood vessels (DV). Arrows mark junction of graft and graft bed. Fibrin deposits are abundant at junction and also extend into the graft bed, upper portions of which also appear necrotic. C and D. 10-d autografts illustrating satisfactory healing, absent inflammatory cell infiltrate, and healthy blood vessels. Junction of graft and graft bed in C, indicated by arrows, is not readily discernible. All 1- μ m-thick, Giemsa-stained Epon sections. A, $\times 125$; B, $\times 160$; C, $\times 30$; D, $\times 500$.

Immunofluorescence Studies. Tissue from allografts and autografts was examined by immunofluorescence at 6–10 d after grafting. The graft-graft bed interface was characterized by brilliant-staining fibrinogen/fibrin deposits in all biopsies studied. Although the antisera employed stained both fibrinogen and fibrin, deposits could be



identified as fibrin in most instances at high magnifications by virtue of their fibrillar structure. In autografts after day 8, fibrin deposits were less extensive than in corresponding allografts, apparently reflecting more-complete healing and fibrin resorption in the former. Only trace quantities of fibrin were deposited elsewhere in autografts or in autograft beds. However, moderate to extensive (1+–4+) fibrin deposits were regularly observed in the intervascular portions of the graft beds of allografts, extending in some instances into the subcutaneous tissue. Less-extensive but similar deposits were observed in intervascular portions of the allografts themselves. This pattern of fibrin deposition is identical to that which has been described in delayed hypersensitivity skin reactions (26, 34). In addition, thrombosed graft vessels in 10-d allografts stained intensely with anti-fibrinogen/fibrin antibodies.

Staining of both allografts and autografts with anti-gamma globulins and anti-human albumin antibodies was entirely negative. Occasional vessels in both allografts and autografts, and their respective graft beds, stained with anti-C'3, but in the absence of associated immunoglobulin deposition, this is a nonspecific finding of unknown significance.

Discussion

The principal new findings are (a) that the extensive epithelial necrosis characteristic of first-set skin allograft rejection was invariably preceded by widespread microvascular damage, particularly affecting the lymphocyte-enveloped venules and arterioles of both the deep dermis of the allograft and the contiguous, superficial graft bed and (b) that the pattern of allograft rejection resembled that of infarction, with extensive microvascular damage and associated greatly increased vascular permeability leading to edema, local hemorrhage, stasis of blood flow, and allograft death due to ischemia. Autografts exhibited neither these vascular changes nor their consequences. It seems probable, therefore, that the microvasculature is the critical target of immunologic damage in first-set skin allografts exchanged among immunologically intact, randomly selected humans. Recent studies of allograft rejection across strong H-2 barriers in the mouse (S. J. Galli, H. J. Winn, and H. F. Dvorak. Manuscript in preparation.) have revealed a similar pattern of microvascular injury and ischemic infarction, indicating that the findings reported here are not peculiar to a single species. Whether similar events accompany the more chronic forms of rejection associated with weaker histocompatibility differences or immunosuppression remains to be determined.

The concept that first-set skin allografts may be destroyed by a process of ischemic infarction is entirely consistent with the well-known observation, here confirmed, that allograft rejection across strong histocompatibility barriers occurs as a relatively sudden event characterized by total and virtually simultaneous necrosis within 24–48 h of all graft and inflammatory cell elements throughout the entire thickness of the graft. Such a pattern of rejection can best be explained on a vascular basis. The concept is also consistent with the careful and frequently overlooked studies of such early workers as Taylor and Lehrfield (35), Converse and Rappaport (36), and Zarem (37), who undertook direct microscopic examination of living grafts in intact animals or man. Lacking the resolution afforded by modern sectioning techniques, these authors were not able to define endothelial cell injury or the nature of graft necrosis but did document an initial phase of graft hyperemia followed by endothelial swelling, local hemorrhage, cessation of blood flow, and rather abrupt graft rejection.

Our findings render less tenable an alternate hypothesis, that allografts are rejected primarily as the result of piecemeal and progressive cytotoxic damage to individual epidermal and other epithelial cells caused by contacts or close associations with infiltrating inflammatory cells. Although scattered lymphocytes and basophils migrated into the graft epidermis and skin appendages, their infiltration appeared to be associated with only focal damage to epithelial cells that could not have accounted for the sudden and nearly simultaneous necrosis of the full thickness of these vascularized skin grafts.

Despite widespread vascular injury, intravascular thrombi were noted only as a late event and predominantly affected graft vessels. Even though the focal endothelial cell damage exhibited by deeper graft bed vessels would have been expected to trigger clotting (38), thrombosis of such vessels was only observed rarely. Graft vessel thrombosis may have contributed to the infarction of allografts. However, it should be remembered that thrombosis is a variable event in clinical situations that involve ischemic infarction of tissue and is not itself a necessary prerequisite for ischemic tissue death.

Although the microvasculature can now be regarded as the critical target in first-set human skin allograft rejection, the mechanisms by which vascular endothelium is damaged remain to be established. Of critical importance to this question is the observation that vessels of both the allograft (presumably of donor origin) and of the graft bed (presumably of host origin) underwent equal and progressive damage. This finding suggests that the final events in first-set skin allograft rejection are not immunologically specific. Members of the lymphocyte series represent the likeliest candidates for the role of effector cells, partly because of their dominant numbers, but also because of their striking anatomic distribution, enveloping many graft and graft bed vessels. Several mechanisms have been described by which lymphocytes may destroy foreign target cells (14). If indeed lymphocytes are responsible for vessel damage, a cytotoxic mechanism that does not require direct and persistent anatomic contacts may be implicated because, except during diapedesis, endothelial cells were separated from enveloping inflammatory cells by the vascular basement membrane and sometimes by perivascular collagen as well. Secretion of a diffuseable mediator selectively toxic for endothelial cells (both donor and host) represents a likely possibility and the susceptibility of endothelial cells to known lymphokines such as lymphotoxin deserves investigation. A role for basophils is also possible because of their accumulation in the days immediately preceding graft rejection and their known capacity to secrete vasoactive mediators such as histamine. Monocytes and macrophages, present in small numbers, might also be implicated. Graft recipients lacked demonstrable antibodies against donor-lymphocyte HLA antigens and immunoglobulin deposits were not observed in vessel walls or elsewhere in rejecting allografts by immunofluorescence, findings that argue against a role for cytotoxic antibodies in the rejection process.

Two objections may be raised to the scheme of allograft rejection proposed here. The first is that many of the morphologic features of allograft rejection closely mimic those of delayed hypersensitivity reactions where epithelial necrosis is not a regular feature. We have recently demonstrated, however, that delayed skin reactions elicited with purified proteins or defined haptens invariably exhibit vascular damage and repair (26, 27). As long as repair keeps pace with injury, tissue necrosis is unlikely to

occur, particularly in an organ as well endowed with vascular anastomoses as the skin. More severe injury to the microvasculature, as may occur in allografts for a variety of reasons (e.g., more prolonged antigen stimulation, qualitative or quantitative differences in the pattern of lymphocyte mediator secretion, etc.), may overwhelm reparative processes, leading to vascular death and stasis of blood flow. In support of this argument, it is well known that severe tuberculin and other delayed hypersensitivity reactions in man and animals exhibit central necrosis; studies of such reactions in the guinea pig reveal a pattern of widespread microvascular damage and cutaneous infarction similar to that observed here (H. F. Dvorak, unpublished data).

A second objection concerns the specificity of allograft rejection. Our data suggest that skin allograft rejection, though induced by immunologically specific mechanisms, is primarily effected by final pathways that damage both foreign and host vessels and cells. Apparently contrary to these findings, other investigators have found a high level of specificity in the immunologic rejection of vascularized allografts. Billingham et al. (6), Billingham and Silvers (39, 40) and Mintz and Silvers (41, 42) employed donor grafts composed of a mixture of genetically compatible and incompatible cells; compatible cells were commonly pigmented melanocytes that could later be positively identified in the recipient. In these experiments, at least some compatible cells generally survived as judged by the persistence of pigmented skin and hair after the rejection of incompatible, nonpigmented skin. Similar experiments have been performed with mixtures of compatible and incompatible epidermal cells and tumor cells with both similar (6, 43) and contradictory (6, 44) results. Critical examination of the published data indicates that they are, in fact, reconcilable with those presented here. Survival of compatible pigment cells in an otherwise incompatible graft may result from migration of such cells from the epidermis or superficial hair follicles of the donor skin to the transected hair follicles of the recipient in the graft bed (45). Because melanocytes are capable of extensive replication as well as migration, survival of only a small fraction of grafted compatible cells could permit the retention of substantial pigmented skin and hair after allograft rejection by a vessel-damaging mechanism that did not itself discriminate between foreign and compatible endothelial cells. Further, Mintz and Silvers (41, 42) found that genetically compatible portions of skin grafts generally did undergo nonspecific rejection if foreign cells comprised a majority of the donor graft. Finally, we do not contend that microvascular damage leading to infarction is the only immunologic mechanism for rejecting foreign cells. Clearly allogeneic cells can be destroyed in a highly specific and discriminating fashion by direct cell contact with cytotoxic lymphocytes or by specific antibodies (14). Although evidence for anti-HLA antibodies was lacking in the present experiments, scattered lymphocytes did infiltrate graft epidermis and appendages and very likely were responsible for the focal epithelial cell dyskeratosis observed after day 5; however, the contribution of this process to graft rejection appeared to be minor. Nonetheless, such an immunologically specific lymphocyte-contact mechanism very likely accounts for the highly selective rejection of incompatible melanoblasts admixed within single hair follicles in grafts of allophenic mouse skin to parental strains (41, 42).

In summary, allograft rejection may involve both highly specific and relatively nonspecific mechanisms operating separately or together, depending on such factors as the nature and antigenicity of the graft, its location, and whether or not it is vascularized. Species variation may also play a role in the pattern of allograft

rejection. That the destruction of foreign cells may be effected by multiple mechanisms is not surprising in view of the well-known heterogeneity of the immune response to relatively simple and better-defined antigens.

Summary

Recent reports of microvascular injury in delayed hypersensitivity skin reactions prompted us to reexamine the pathogenesis of first-set skin allograft rejection in man using morphologic techniques that allowed both extensive vessel sampling and unequivocal evaluation of microvascular endothelium. We here report that widespread microvascular damage is a characteristic, early consequence of the cellular immune response to first-set human skin allografts and is qualitatively similar to, but substantially more extensive than, that occurring in delayed hypersensitivity reactions. Microvascular damage invariably preceded significant epithelial necrosis and affected initially and primarily those venules, arterioles, and small veins enveloped by lymphocytes. Vessels of both the allograft itself and the underlying graft bed (recipient tissue) were equally affected. These data suggest that endothelial cells of the microvasculature are the critical target of the immune response in first-set vascularized skin allograft rejection in man and that rejection can be attributed largely to ischemic infarction resulting from extensive microvascular damage. Other mechanisms, such as direct cellular contacts between infiltrating lymphocytes and epithelium, apparently played only a minor role.

The findings presented here indicate that the rejection of first-set vascularized skin allografts, though induced by immunologically specific mechanisms, is primarily effected by final pathways that are relatively nonspecific and that may cause damage to both foreign and host vessels and cells. Rather than contradicting studies demonstrating the exquisite specificity of allograft rejection in other systems, these findings provide a further example of the heterogeneity of the cellular immune response. Recognition of the critical role of immunologically mediated microvascular injury may prove important both for an understanding of the biology of allograft rejection and for strategies aimed at prolonging allograft survival.

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