Lymphology 14 (1981) 81–85

Alymphatic Pedicles

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Host recognition of transplanted foreign tissue, the afferent limb of the immune reflex, depends, in part, on the method used to expose the host to the allogeneic antigens. For large immediately vascularized organs, such as transplanted kidneys, it is logical to assume that sensitization occurs through the leakage of antigenic material, in the form of cellular components or fragments, into the venous effluent from the graft. This establishes contact with the host's central lymphoid tissue directly or through reticulo-endothelia system processing (1). Another important mechanism, suggested by *Strober* and *Gowans* (2), is "peripheral sensitization", which allows for recognition of allograft antigens by sensitive host cells as they come into contact with the endothelial surfaces of the graft. These "sensitized" lymphoid cells or macrophages, or both, then initiate a cascade of recognition steps which expands a population of effector cells capable of the ultimate destruction of the allograft. Donor leukocyte passengers in the intravascular and interstitial compartments of the allograft may also reinforce the antigenic challenge (3).

For tissues which do not initially confront the host via a mechanism of immediate vascularization, but which are implanted as "free grafts" such as skin, tumor, and endocrine allografts, an initial "healing-in" process is required. For these types of grafts, the critical importance of the lymphatic circulation has been firmly established. Much of our knowledge of immunologically privileged sites stems from manipulation of the lymphatic circulation in recipients of freely transplanted allografts. Initial assumptions that lymphatics draining allograft beds carry antigenic material were based on experiments in which cytologic and adoptive transfer studies pointed to the local draining nodes as the seat of the immune response (4). Despite this, excision of the regional lymph nodes was found to prolong the survival of skin allografts by only a few days in mice, dogs, guinea pigs, and man. Construction of a totally alymphatic bed transplant site, however, does strikingly prolong the survival of allografts.

An artifically privileged transplant site was first devised by *Barker* and *Billingham* (5) who surgically separated a pedicle of skin from the flank in guinea pigs. A single vascular "umbilical cord" and the underlying subcutaneous tissue and panniculus carnosus muscle were preserved in the dissection to maintain viability of the skin pedicle. The injection of Patent Blue V dye into the flap failed to demonstrate any connection between the lymphatic network in the skin of the flap and draining regional lymphatics of the host. The operative skin defect was sutured around the vascular pedicle and the flap was placed in a plastic capsule with an opening provided for the umbilical cord. This capsule (made from a small petri dish with a radial groove extending from the center to the edge) was then firmly fixed to the skin with glue and circumferential dressings (Fig. 1).

Guinea pig ear skin allografts transplanted across a major histocompatibility barrier and placed in shallow beds cut into skin pedicles were not rejected, remaining viable and healthy,

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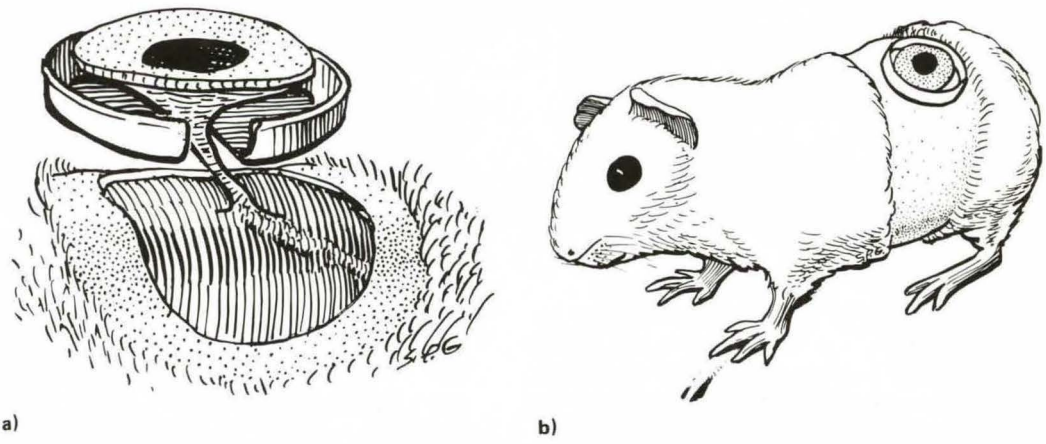

a) b)

Fig. 1 A lymphatic skin flap on guinea pig flank (a). Petri dish interferes with direct contact between flap, (containing an intraflap skin allograft) and underlying lymphatics of host when placed directly over healing donor site (b)

as evidenced by continued hair growth, and surviving as long as the flaps themselves remained viable (20–100 days). This was in contrast to orthotopic skin allografts in conventional transplant sites exchanged between the same two strains, the survival of which was 8–10 days. In addition, subsequent orthotopic skin grafts from the original donor were not rejected in an accelerated manner by animals with intraflap allografts indicating that the latter had no sensitized their hosts. Intraflap grafts were rejected rapidly, however, if the recipients had been specifically presensitized to donor strain skin or if the hosts underwent active or adoptive immunization after the intraflap graft had been in place. Thus, the efferent limb of the immune reflex remained intact. Furthermore, various methods used to preserve or reconstitute lymphatic pathways from flap to host (such as leaving intact a narrow skin bridge, dissecting the flap from axillary skin containing a large accompanying lymphatic vessel, or reestablishing continuity by suturing the flap to a fresh skin defect) led to ultimate rejection of previously "protected" skin allografts.

The immunologic privilege provided skin allografts in these experiments was also shown to extend to other tissues. Using a similar skin flap model, *Futrell* and co-workers (6) found that 6×10^6 cells from a methyl-cholanthrene-induced sarcoma would result in small tumors which were eventually rejected when injected into syngeneic guinea pig hosts. The same number of cells injected to an alymphatic pedicle led to progressive tumor growth and death of the animal. However, unlike intraflap skin grafts, tumors growing on alymphatic pedicles seemed to sensitize their hosts within 20 days. Evidence for this came from the failure of secondary tumor grafts in these animals. Theoretically, the embolization of antigenic fragments of the tumors via the draining vein may have sensitized the hosts, although there seemed to be no evidence of distance metastases. Another explanation is that peripheral sensitization ensued.

A similar but less technically exact method of isolating an area of skin from the lymphatic circulation was also developed by *Barker* and *Billingham* (7). (Fig. 2c) In guinea pigs or rats island of skin, 1.5 x 1.5 cm, were allowed to remain undisturbed in the center of a large rectangular bed of raw panniculus carnosus muscle from which the epimysium and its contained lymphatics had been removed. For 18 days after the creation of the island, Patent Blue V dye failed to enter the lymphatics and the regional nodes. Skin allografts

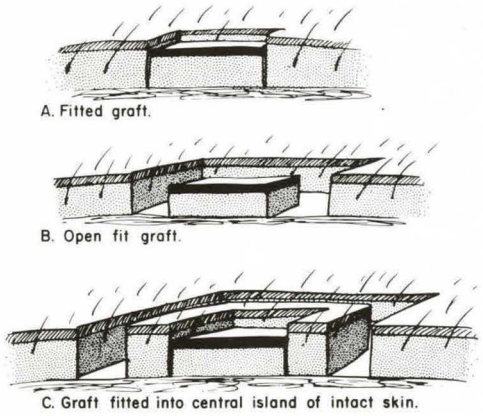

Fig. 2 Skin grafts placed in fitted bed rapidly develop connections with host lymphatic system and are thus rejected in the usual manner (a). Prolongation of graft survival can be achieved by creating a situation which delays lymphatic reconstitution such as the open fit graft (b) or the skin island (c)

transplanted to these islands were initially accepted and often enjoyed very prolonged survival prior to their eventual rejection (18–> 50 days).

Ziegler and co-workers (8–10) also used the skin island model to demonstrate that allografts of tumor cell suspensions, rapidly rejected if transplanted to intact flank skin, would flourish for much longer periods before rejection if placed in the alymphatic islands. (Tumor regression times were 7.3 and 9.9 days in flank skin of AgB compatible and incompatible hosts compared to skin island tumor survivals of 42.1 and 22. 3 days respectively). Syngeneic tumors on skin islands were noted earlier, grew more rapidly, and killed the recipients sooner if injected into skin islands. In another series of experiments performed by *Naji* and *Barker* (11), skin islands were also able to prolong the survival times of parathyroid allografts transplanted across both minor and major histocompatibility barriers.

A final method of interfering with lymphatic continuity between donor and host is the "open fit" skin graft bed (Fig. 2b). The technique, described by *Barker* and *Billingham* (7) involves definition of the limits of the prospective graft bed with a knife followed by avulsion of the skin from the underlying tissue. This maneuver denudes the panniculus carnosus muscle of its superficial fascia and underlying lymphatics if the guinea pig or rat, but not the rabbit, is used for the experiment, since in the first two the skin is tightly applied to the epimysium of the underlying muscle. The survival times of skin allografts placed on large open beds such as those described were approximately twice those of the control grafts fitted into beds which allowed for perfect fit and thus the rapid re-establishment of lymphatic continuity. "Open fit" grafts, transplanted eccentrically, so as to contact the host along one margin, or placed in previously sensitized animals, were rejected in the usual time.

It can be concluded that grafts which undergo delayed reconstitution of vascular and lymphatic reconstitution with the host, such as skin, endocrine and tumor allografts, can be protected to varying degrees by prolonging the time required for the development of lymphatic continuity. The extend of prolongation is directly related to the timing of the lymphatic growth, but, in the case of the alymphatic pedicle, it may be indefinite.

Studies of the kind reviewed above stimulated interest in attempts to discover the differences between skin and whole organ grafts in terms of their ability to survive the allograft reaction (12–15). It is known that skin grafts are more rapidly rejected than immediately vascularized grafts such as heart or kidney transplanted with the same strain combination and a number of reasons have been suggested. Skin may be intrinsically more sensitive to ischemia than other organs and the critical 3–4 day healing period required for revascularization may either damage the skin to the point that it is more susceptible to rejection, or render the allograft more immunogenic by facilitating the release antigenic material from the damaged tissue. Further, as an orthotopic skin graft is transplanted to a bed rich in lymphatics so that access to the lymphatic system of the host is rapidly

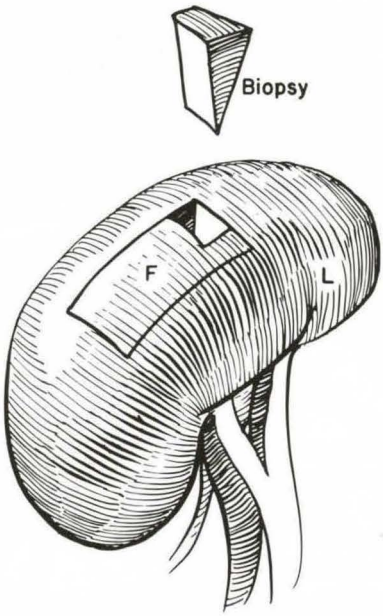

Fig. 3 Composite skin-kidney graft created by transplanting Fischer rat ear skin to the surface of the kidney of a tolerant host. Biopsy assures skin viability. When transplanted, the composite graft provides immediate vascularization to the skin and kidney simultaneously

established and may provide for earlier recognition of antigenicity than may be seen with vascularized organs. Another theory arises from the assumption that epidermal cells express alloantigenic specificities not found on leukocytes and that these "SK" antigens can function as histocompatibility antigens (16, 17). There is considerable variation in the expression of SK antigens among different inbred rodent strains (17). Nevertheless, there are clearly other differences between skin and other organs, since, in experiments in dogs (18), sheep (19), and man (20), prompt rejection of renal allografts occurs despite attempts to isolated the grafts from the lymphatics of the host.

Several workers have attempted to examine whether the unusual susceptibility of skin grafts to rejection is entirely dependent on their mode of transplantation by transplanting skin allografts in the same manner as immediately vascularized whole organs. Both *Salyer* and *Kyger* (21) and *Cho* et al. (22) have shown that skin allografts based only on the inferior epigastric artery and vein of the host were rejected in the same time period as orthotopic grafts in AgB compatible and incompatible strains. A theoretical objection to these experiments is that the periphery of skin grafts were sutured to the host skin thus allowing for a small but definite contact with the host lymphatics.

Wustrack et al. (23) created four rat models in which the rates of lymphatic and vascular reconstitution varied. Transplantation of skin, devoid of lymphatic drainage and nourished only by a single artery and vein, resulted in survival times similar to conventionally placed orthotopic skin grafts in the same strain combination (8.6 and 8.1 days respectively). It was only when small grafts were placed within these pedicles and protected from lymphatic reconstitution ("delayed vascular; delayed lymphatic") that prolongation of skin graft survival to 19 days was noted.

To avoid the possibility that the immediately vascularized skin allograft in the above experiments was damaged by nonspecific ischemic factors, *Perloff* and *Barker* (24) Fig. 3) developed a composite skin-kidney allograft whose vascular reconstitution was immediate. Fischer rat skin was transplanted to the raw surface of the kidneys of two month old Lewis rats having been rendered tolerant by an inoculum of 30×10^6 Fischer lymph node cells administered within the first 24 hours of life. After one month, the kidney, with its associated skin allograft (protected by the tolerant state of the host) was transplanted by routine microvascular anastomoses to Lewis recipients (syngeneic to the kidney but allogeneic to the skin). For five days after transplantation the skin and kidney appeared histologically normal. From the sixth to the eleventh day an active inflammatory response was seen at the skin-kidney interface, and, by 12 days, rejection of the skin was complete. Thus an immediately vascularized skin allograft with connections to the host which were identical to a renal allograft was rejected in the same

period of time as an orthotopic skin allograft in a weakly histoincompatible strain combination in which renal allografts usually survive for many weeks. A theoretical disadvantage of this protocol is that passenger leukocytes from the chimeric skin-kidney donors may have sensitized the recipients to the skin allograft portion of the composite graft.

The technics described above allow for further investigation of the influence of lymphatics on enhancement techniques and the further evaluation of privilege as it applies to various tissues as well as different sites within a given host.

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