CELLULAR AND VASCULAR COMPONENTS OF THE ALLOGRAFT REACTION

EVIDENCE FROM RETURNED SKIN ALLOGRAFTS*

BY PETER B. LAMBERT, M.B., CH.B. AND HOWARD A. FRANK, M.D.

(From the Department of Surgery, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts 02215)

(Received for publication 8 June 1970)

The placing of a skin graft on an allogeneic host excites a complex group of interactions which culminate in the establishment of specific immunity in the host and the destruction of the graft. A wealth of descriptive information on graft destruction is available but knowledge of the mechanisms involved is limited.

Additional insight might be gained by returning the graft to its original donor at a selected stage prior to graft destruction, so that the participation of the intermediate host is terminated and any continuing processes take place within the autologous environment provided by the original host. Immune interactions are not thereby terminated, however, for immunologically competent allogeneic cells, some already activated, derived from the intermediate host are transferred with the returned graft. The contributions of these cells may be further examined by (a) suppression of the immune response in the intermediate host during the period of graft residence, or (b) sensitization of the original donor to the intermediate host before return of the graft with its transferred cells. Experiments along these lines are here described.

A number of factors in addition to the allogeneic cells transferred from the intermediate host may prevent such returned grafts from behaving like simple autografts. Epithelial, fibroblastic, and vascular proliferation, which will have occurred during the allograft phase, may modify healing following retransfer, and grafts left on the intermediate host for too long may have experienced destructive changes too severe to permit survival. The possibility of histocompatibility change during the period of residence as an allograft also exists: allogeneic RNA is said to modify acceptance of skin grafts (1, 2); nuclear material which exchanges locally between cells of graft and host (3, 4) might transfer genetic information; incubation with allogeneic skin is reported to prevent the acceptance of skin isografts (5). The experiments here described examine only the initial reacceptance (4 days) following replantation and are therefore influenced only by factors operative in this early period; the data to be presented suggest that the events of this period are predominantly influenced by the presence of the transferred allogeneic cells.

Replantation experiments reported by others have been designed to answer somewhat different questions. Medawar returned allografted skin to the donor rabbit in

* Supported by National Institutes of Health Grant GM 08772.
order to determine the remaining viability at given stages of the rejection process (6). Edgerton determined in mice that skin grafts, although initially reaccepted, would not permanently survive replantation if they had been allowed to remain on the intermediate host more than 2 days before expected rejection (7). Silvers et al. replanted female isografts from male recipients to female animals of the donor strain in order to examine possible connections of the Y chromosome and male hormone with the antigenicity of male skin (8). Steinmuller demonstrated by replantation to isogeneic rats that skin resident for long periods of time on animals made adoptively tolerant at birth underwent an antigenic change; he attributed this change to the presence in the skin of cells of the original tolerance-conferring inoculum (9), but this could also have been caused by some of the mechanisms listed above. Brautbar et al. have recently described nonsurvival of replanted rat skin after only a 24 hr sojourn on an allogeneic intermediate host (10), a finding which is unexpected in view of the experience of others, and of his own finding of survival after a 48 hr sojourn. Replantation experiments using kidneys show a limiting interval of intermediate host residence which according to Dempster (11) is between 30 and 72 hr, but which in the immunosuppressed host (12) can be extended indefinitely. Clark et al. demonstrated the destruction of replanted kidneys after only a few hours in presensitized, X-irradiated intermediate hosts, and attributed this destruction to the action of humoral antibody in the putative absence of transferred cells (13).

In the present experiments, skin grafts were allowed to remain on the intermediate host for 4 days. This period is long enough to produce an immune response but is 2 days short of the mean time for allograft rejection in these pairings. The grafts were then returned to the original donor and were studied from the standpoint of the initial acceptance of the graft, the reaction of the cognate lymph node, and the modifications brought about by specific sensitization or immune suppression. We chose the rabbit for these experiments, in spite of the unavailability of genetically pure strains, because the rabbit ear presents both a unique opportunity for vascular studies and a quantifiable regional lymph node.

Materials and Methods

Young rabbits, about 4 lb in weight, were used. To avoid inadvertent littermate pairing, the animals were either from two different strains (New Zealand white and Dutch belted) or were New Zealand whites obtained from different breeders. The rejection time of standard skin allografts was determined for each combination.

Experimental Groups.—In group A, a disc of skin from the dorsum of the ear of one rabbit was grafted onto the ear of another, the defect on the donor ear being covered with an autograft from the opposite ear. In group B grafts were exchanged between the two members of each pair so as to sensitize each donor to the intermediate host; the opposite ear remained untouched.

4 days later (or in subgroups, 3 or 5 days), the allograft was carefully detached from its bed and resutured to its site of origin after removal of the graft covering the initial skin defect (autograft in group A and allograft in group B). In some experiments the allograft was returned to a freshly prepared bed. The autograft was returned to its site of origin after removal of the crust that had formed on the open wound.
COMPONENTS OF THE ALLOGRAFT REACTION

In a number of pairs within group B, one intermediate host of each pair was made immuno-logically unresponsive by twice-daily injection of dexamethasone (2 mg). An unidentified spontaneous diarrheal illness which led to weight loss, marked atrophy of lymphoid tissues, and poor or no transplantation-immune responses was taken advantage of by using these rabbits, also given dexamethasone, as immune-suppressed intermediate hosts in several experiments.

Final studies of the graft and lymph node were carried out 4 days after replantation.

Techniques and Observations.—Grafting was done under nembutal and ether anesthesia. Full-thickness skin discs, 1.7 cm in diameter, from the dorsum of the ear were excised by sharp dissection and sewn in place with 6-0 silk.

The grafts were inspected daily and the regional lymph node at the base of the ear palpated and graded in size on a 4-point scale. At the end of the postreplantation period (4 days), sections of the grafts and graft beds were prepared for histological study (hematoxylin and eosin stain). Graft cells obtained by scraping were smeared and stained with Wright’s stain. The draining lymph nodes were dissected clean and weighed and their weight ratios with the node of the opposite ear established; lymph node smears (Wright’s stain) and sections (H and E stain) were studied histologically.

Microangiographic Study.—Carried out postmortem following the filling of the vasculature of the ear with a radio-opaque medium (20% Micropaque in 10% Formalin) by injection via the cannulated central artery. The ears were cut by hand transversely through the graft in 3 mm sections, the cut surfaces of which were placed in contact with Kodak Fin Grain Positive film (Eastman Kodak Co., Rochester, N. Y.) and exposed to a 25 kv, 18 ma X-ray beam at a focal spot to object distance of 18 in. Animals were sacrificed for these studies 2 days after replantation.

Radioautographic Study.—Carried out following the injection of tritiated thymidine: (a) intradermally (5 μCi in 0.05 cc saline) into the returned grafts 2 hr before sacrifice at 2 days after grafting; (b) intravenously into the final host (0.75 μCi/kg bodyweight) 2 hr before sacrifice 2 days after graft reimplantation; (c) intravascularly (1 μCi/kg) into the final host 2 hr before reimplantation, the graft examined 3 days later; and (d) intradermally (5 μCi in 0.05 cc saline) at day 4 into each of two identical allografts on the intermediate host, 2 hr before the grafts were detached and one of them was returned to the original donor and the other fixed in formalin. The returned graft was studied at 2 days after return. Radioautographs of sections of grafts and draining lymph nodes were prepared by the dipping technique, using Kodak NTB-2 emulsion, exposed for 2- and 8 wk, and stained with hematoxylin and eosin.

RESULTS

Control Autografts and Allografts

Control autografts, lifted and retransferred by the techniques described, survived regularly. They were thicker and more contracted than primary autografts and presented an uneven surface occasionally focally eroded. The range of allograft-rejection time in the pair combinations of these experiments was 5–8 days. At 4 days the allografts were uniformly healthy in gross appearance. They were moderately thickened, pinkish in color, and they bled briskly on detachment. Histological examination disclosed moderate mononuclear cell and granulocytic infiltration, sometimes perifollicular in arrangement (Fig. 1a). Radioautographs after intradermal injection showed extensive labeling of the basal layer of the epidermis and of the endothelial
FIG. 1. Photomicrographs at equal magnification of (a) a 4 day allograft, showing moderate cellular infiltration of the dermis of the graft but no invasion of the epidermis, and (b) a 4 day autoallograft 4 days after its return to the donor, showing a marked increase in the cellular infiltration. Many of the new cells are of host origin. Epithelial thickening has progressed to a degree rarely seen in a primary allograft. Incipient epithelial invasion and destruction by cells of the infiltrate is evident here, however, (see Fig. 8) as occurs later in a simple 5 day allograft (see Fig. 6). Scale: 0.1 mm.

FIG. 2. Radioautographs of a 4 day allograft (a) and of the same skin as an autoallograft 2 days later (b). Both were labeled by local injection of tritiated thymidine 2 hr before removal and fixation (a) or replantation (b).

The marked proliferative activity of allograft epidermis is indicated by the extensive uptake of label by the basal cells. The reacceptance and viability of this graft are attested to by the proliferation of its epidermal cells, shown by the dilution of label in the basal layer and the progression of labeled cells through all layers of the epidermis, including the stratum corneum. Cell accumulation within the dermis is still slight at 2 days. Scale: 0.1 mm.
cells and fibroblasts of the dermis (Fig. 2 a); a few of the mononuclear cells of the infiltrate also took up the labeled thymidine. Both the infiltration and the labeling were in excess of that seen in autografts at 4 days.

Returned Allografts (Autoallografts)

Normal recipients.—Retransplanted allografts, now autografts, on normal (nonsensitized) original donors were usually reaccepted (Table I and Fig. 3). Vascular connection between graft and host was demonstrated at 2 days by gross observation, by microangiography (Fig. 4 a), and by radioautographs which showed labeled endothelial cells within the graft following injection of the H3Th either intradermally into the graft (Fig. 5) or intravenously into the recipient.

Focal epithelial erosion and infiltration was common in the autoallografts at 4 days, as in the later stages of simple allografts (Fig. 6), but the noneroded epithelium was actively proliferating and had thickened considerably (Figs. 7a, 1b, and 8).

An extensive cellular reaction had developed within the dermis at this time, considerably more intense than that present when it was returned from the intermediate host (Fig. 1b). The predominant cell types were granulocytes and

### TABLE I

**Acceptance of Autoallografts in Nonsensitized and Sensitized Hosts (Original Donors of the Grafts), as Judged by Gross Observation**

| Host nonsensitized | Day 1 | Day 2 | Day 3 | Day 4 | Host sensitized | Day 1 | Day 2 | Day 3 | Day 4 |
|--------------------|-------|-------|-------|-------|----------------|-------|-------|-------|-------|
| Experiment No. | | | | | Experiment No. | | | | |
| 27 | 1 | 0 | 0 | 0 | 17 | 1 | 0 | 0 | 0 |
| 36 | 1 | 1 | 1 | 1 | 19 | 1 | 0 | 0 | 0 |
| 37 | 1 | 1 | 1 | 1 | 25 | 0 | 0 | 0 | 0 |
| 47 | 2 | 2 | 1 | 0 | 26 | 0 | 0 | 0 | 0 |
| 64 | 2 | 2 | 1 | 1 | 49 | 1 | 1 | 1 | 1§ |
| 103 | 1 | 1 | 1 | 1 | 50 | 1 | 1 | 1 | 1§ |
| 113 | 2 | 2 | 1 | 2 | 68 | 0 | 0 | 0 | 0 |
| 117A | 2 | 2 | 2 | 2 | 72 | 0 | 0 | 0 | 0 |
| 118A | 2 | 2 | 2 | 2 | 73 | 0 | 0 | 0 | 0 |
| B79 | 2 | 2 | | | 87 | 0 | 0 | 0 | 0 |
| B79 | 2 | 2 | | | 117B | 0 | 0 | 0 | 0 |
| B87 | 1 | 0 | 0 | 0 | B87 | 1 | 0 | 0 | 0 |
| B89 | 0 | 0 | 0 | 0 | B89 | 0 | 0 | 0 | 0 |

* SCALE: 2 = viable; 1 = probably viable; 0 = dead.
† Day after replantation.
§ One intermediate host in this pair responded weakly to the immunizing graft.
FIG. 3. Photographs of 4-day old allografts, 2 days (a) and 4 days (b) after return to non-sensitized (left), and sensitized (right) donors. 2 days after its return the graft on a normal host (left) looks healthy, whereas on a sensitized host (right) it is already escharotic; by 4 days, surface erosions attest to the intense cellular activity within grafts on nonsensitized hosts (left), but a substantial part of the surface remains well epithelialized and healthy: a comparable graft on a sensitized animal (right) is dry throughout its thickness and ready to be cast off. X2.

FIG. 4. Microangiograms of 2-day old autoallografts on a nonsensitized (a) and sensitized animal (b). Full vascularization of the one (a), and the absence of vascular connection in the other (b) are evident. (The arrows mark the graft limits.) Scale: 1 mm.
FIG. 5. Radioautograph of an autoallograft 2 days after its return to the nonsensitized donor, labeled by the local injection of tritiated thymidine 2 hr before sacrifice of the animal. Epithelial proliferation is noted as in Fig. 2 b. Endothelial cell proliferation within the dermis of the graft, clearly superficial to the graft-host interface (not shown), is also indicated by the uptake of label. Functional continuity with the blood vessels of the host is shown by the filling of graft vessels with a radio-opaque mass (arrow) injected into a host artery (See microangiogram, Fig. 4 a). Scale: 0.1 mm.

FIG. 6. Photomicrographs of a 5 day old allograft. (a) Medium-power view showing an increased cellular infiltration of the dermis (compare with Fig. 1 a), with focal invasion of the epithelium (arrow). (b) High-power view of the site of epithelial invasion by inflammatory cells, mostly granulocytes and histiocytes. Degranulation of invading cells is seen both within the epidermis and at the epithelial-dermal junction. The process of invasion of the proliferating epithelium followed by epithelial destruction appears identical to that observed in the autoallograft on its autologous host (Fig. 8). In neither case is a vascular component of the destructive process evident at this stage. Scale: Fig. 6 a, 0.1 mm; 6 b, 0.01 mm.
Fig. 7. Photomicrographs at equal magnification of autoallografts, 4 days after their return to the donors. (a) In the nonsensitized animal the graft is reaccepted although a portion of the epithelium has become eroded in consequence of the cellular reaction within the graft. (b) In the animal sensitized to the intermediate host, the graft is cast off as a full-thickness hemorrhagic slough. Scale represents 0.5 mm.

Fig. 8. High-magnification photomicrograph of the epidermal-dermal junction of an autoallograft 4 days after its return to a nonsensitized host, showing encroachment by inflammatory cells on the epithelium. Epithelial cells are proliferating in the immediate vicinity of a local necrotizing reaction involving granulocytes, some degranulated and disintegrated, and histiocytes; it would appear that the cell infiltration is not secondary to an already dead epithelium, but is the cause of the necrosis subsequently observed. Scale represents 0.01 mm.
histiocytes; small lymphoid cells and blast cells, many in mitosis, were also present. Lymph spaces were distended with small lymphocytes, histiocytes, blast cells, and with macrophages containing ingested chromatin (Fig. 9). The histological pattern, including a marked perifollicular concentration of cells, was characteristic of a transfer reaction. Radioautographic examination of the graft on a prelabeled host demonstrated labeling of many of the cells of the infiltrate, indicating that they were of host origin. Smears of scrapings of the graft undersurface and the graft bed disclosed many granulocytes, a heterogeneous lymphoid cell population, including blast cells, and tissue mast cells and fibroblasts. Radioautographs following intravenous or intradermal injection of H$_3$Th at 2 days after replantation demonstrated extensive labeling of epithelial cells and of blast and endothelial cells in the dermis.

Epithelial proliferation within returned allografts was evident in radioautographs prepared 2 days after their return prelabeled to the primary host (Fig. 2 b). Considerable transfer of label from graft to host cells was also demonstrated.
Sensitized Recipients.—The result was strikingly different when similar allografts were returned to original donors which had in the interval been sensitized to the intermediate host. In the sensitized group, with the exception of a single pair in which the sensitization response was weak, no graft was re-accepted. Nonviability was usually apparent by 2 days (Fig. 3) and sometimes by 24 hr after replantation (Table I). All of these grafts were necrotic by the 4th day and were cast off as a full thickness slough (Fig. 7b).

Microangiographic, histologic, and radioautographic studies at 2 days showed no vascular connections (Fig. 4 b), no cellular influx, and no labeling of endothelial or epithelial cells of the graft following intravenous injection into the host, although some epithelial cells were still alive as shown by their incorporation of labeled thymidine introduced intradermally into the graft. These gross and histologic findings were no different in grafts returned to freshly prepared beds instead of the original beds which had meanwhile carried an allograft.
Radioautographs of prelabeled grafts showed scattered clusters of labeled material within necrotic cells and also in extracellular spaces, but no evidence of epithelial proliferation and also no uptake by host cells of labeled material.

*Immunologically Suppressed Intermediate Hosts.*—Allografts remained thin and pliable on intermediate hosts spontaneously anergic in their immune response and/or treated with dexamethasone; the draining lymph node showed no sign of activation. When returned to the original hosts, these grafts were accepted like unmodified autografts, even though the original host had been sensitized to the intermediate host (Fig. 10).

![Graph](image)

*Fig. 11.* Weights of lymph nodes draining returned allografts, 4 days after replantation, and ratios of their weights to that of their contralateral control nodes in normal and sensitized animals. The differences of the means in these 2 groups are significant at the $P<0.01$ level.

**Regional Lymph Nodes**

*Normal Recipients.*—In nonsensitized hosts, the node draining the ear to which an autoallograft was returned enlarged markedly and promptly, reaching approximately four times its normal weight by the 4th day (Figs. 11 and 12). This compares with an approximately 30% enlargement of nodes draining unmodified autografts and an approximate 2 to 3-fold increase in weight of those draining comparable allografts. The node enlargement excited by the autoallograft increased with the period of residence on the intermediate host (3–5 days).

Smears of the enlarged nodes draining returned autoallografts showed very large numbers of immunoblasts, many in mitosis, and many macrophages and polymorphonuclear neutrophils in addition to the usual lymph node cell population. The macrophages were considerably more numerous than in smears from normal nodes, and many contained ingested red cells or chromatin ma-
terial. Such chromatin droplets sometimes contained label in radioautographs made after "flash-labeling" with H3Th 2 hr before sacrifice. Occasionally very large cells with basophilic birefringent granules and a rim of basophilic material were seen. They were of undetermined type, but would seem to have reached the nodes from the grafts, for scrapes of graft undersurfaces sometimes demonstrated similar cells.

Histological sections of these nodes showed a considerably expanded paracortical area and medulla. Follicular areas were confluent at the afferent pole. In the medulla, sinusoids lined with flat endothelium were filled with blast cells and macrophages containing ingested red blood cells; vascular spaces lined with tall cuboidal cells contained small lymphocytes and many granulocytes. Portions of the medulla had a fleshy appearance due to the accumulation of histioreticulocytes, resembling the spleens described by Armstrong et al. in connection with lymphomas arising following the graft-versus-host reaction. (14)

Sensitized Recipients.—In this group of experiments, the ear which received the returned allograft had carried a sensitizing allograft from the intermediate host during the interval prior to the return of the autograft. The lymph node at the time of replantation was therefore enlarged to the size of a 4 day allograft-draining node. The striking finding in all experiments of this group was the definite shrinkage of the node when the allograft was replaced by an auto-allograft (Figs. 11 and 12). Histological sections disclosed activated nodes, but the smears of these nodes showed decidedly fewer hemocytoblasts and many fewer mitotic figures than did nodes from nonsensitized hosts.

Immunologically Suppressed Intermediate Hosts.—Grafts returned from
COMPONENTS OF THE ALLOGRAFT REACTION

intermediate hosts which, because of dexamethasone treatment or spontaneous illness, had manifested no allograft response excited no significant lymph node enlargement when returned to the nonsensitized original donor. The already enlarged lymph nodes of the sensitized donors remained large.

DISCUSSION

Skin grafts returned to the unmodified original donor after a 4 day sojourn on an intermediate host were reaccepted, as evidenced by vascularization of the graft and by continuing epithelial and endothelial DNA synthesis and proliferation. However, an intense cellular reaction was engendered in the graft and in its draining lymph node, a reaction considerably more intense and rapid in development than the response to a primary allograft in a comparable time span. This reaction, which resembled in all respects an induced transfer reaction (15, 16), did not occur unless the intermediate host was immunologically competent; it seems clearly attributable to the allogeneic cells of the intermediate host, particularly those already activated by antigen, within the returned graft. Epithelial destruction ensued, even though the skin had been returned to its native habitat; further graft injury would probably have occurred in subsequent days until the transfer reaction had terminated.

The epithelial necrosis did not occur during the allograft period, for the epithelium was demonstrably viable and free of cellular infiltrate upon return to the original donor; nor was this necrosis due to histocompatibility change, for even an allogeneic tissue would not have been expected to be destroyed so quickly by a nonsensitized host. The histological evidence points strongly to the granulocytic and histiocytic infiltration as the effector mechanism of epithelial destruction. In the returned allograft most of these cells are autologous; therefore, if these are in fact the effector cells, the tissue destruction is the nonspecific end result of the interaction between the specifically sensitized allogeneic cells within the returned graft and the homologous antigen of the final host. Similar nonspecific destructive changes induced by histocompatible lymphoid cells in the kidney have also been described (17).

The rapidity of the accumulation of granulocytes, histiocytes, and blast cells within the replanted graft may have been partly due to the faster re-vascularization of returned grafts (7), but more decisively to the chemotactic effect of cells already antigenically activated within the ambience of the homologous antigen. The progressive cellular accumulation in returned allografts seemed equal to or in excess of that seen later in allografts allowed to proceed to destruction on the allogeneic host. The chemotactic stimulus is apparently part of a self-sustaining local reaction which is independent of the systemic immunization of the host. The requirements for the initiation and

1 Lambert, P. B., Manuscript in preparation.
support of this reaction appear to be the presence of immunologically activated lymphoid cells in the specific antigenic environment, a pool of nonspecific cells, and vascular connections which make the pool available; all of these are present in both the autoallograft and the simple allograft. In the autoallograft this process is augmented by a concurrent mixed leukocyte reaction, which adds a strong mitogenic stimulus to the chemotactic one, with one reinforcing the other. The interaction is self-limiting, however, in the returned graft, by virtue of the eventual destruction of the transferred allogeneic cells, either specifically by the host’s immune mechanisms or as part of the local nonspecific destructive process. Large portions of returned allografts may, therefore, survive. In the unmodified allograft, however, the cellular activity, augmented in later stages by the development of humoral antibody, proceeds unchecked to the destruction of the graft.

One may choose to consider the returned allograft reaction, in its early stage, to be an example of a local graft-versus-host reaction, in as much as the immunologically activated cells are now the foreign cells. The use of the term simply on the basis of locale, however, neglects underlying mechanisms and may therefore be inaccurate. Since the final effectors of the destructive reaction would seem to be nonspecific host cells, a graft-versus-host reaction can not be defined in terms of effector cells either, except in the special case of the radiation chimera, all of whose relevant cells are derived from grafted allogenic stem cells. The most meaningful definition of graft-versus-host or host-versus-graft reaction would therefore be in terms of the activated cells which, upon contact with the homologous antigen, become the source of various mediator substances (for which the useful term “lymphokines” has recently been suggested [18]), which in turn may cause the nonspecific destructive intervention of host cells. However, in immunologically competent recipients it is at present impossible to distinguish the contribution of grafted cells from that of activated host cells. A graft-versus-host reaction can therefore be accurately identified only in the situation of a tolerant or unresponsive host; this was, of course, the circumstance in which the term was first used as an explanation for the mechanism of “runt or homologous disease” (19, 20); its use is best reserved for this unequivocal situation.

The cell population of a 4 day old allograft and that of its draining lymph node would appear to be identical as judged by morphological and staining criteria, and by the intense and similar reaction each evokes when transferred to the immunologically homologous host, in the one case by the replantation of the graft, in the other by the intradermal injection of cell suspensions prepared from the lymph node (transfer reaction). Such studies of ordinary allografts do not establish whether the cellular accumulation occurred primarily in the graft or in the node. The morphologic evidence and the sequence of events in the returned allograft preparation seem to indicate a primary cellular ac-
cumulation at the graft site, with subsequent migration to the regional node. This sequence of events supports the concept of peripheral sensitization. The great size of the autoallograft-draining lymph node, however, suggests a further local accumulation within the node, in response to the same chemotactic, mitogenic, and probable migration-inhibitory factors operative there.

Sensitization of the primary host to the intermediate host strikingly altered the course of events following graft return. In this circumstance, the grafts were never reaccepted. Vascularization did not occur. The tempo of rejection was not that of a primary allograft, but that of an accelerated or white-graft reaction. Cellular infiltration was absent, undoubtedly mainly because of a lack of vascular connection. Similarly, the lymph node draining this ear, activated during the intermediate period by the sensitizing allograft, decreased in size upon return of the graft, presumably because of the absence of the cellular reaction at the graft. The antigenic effect of the graft was clearly due to the contained cells of the intermediate host, since such grafts were reaccepted like unmodified autografts when returned from intermediate hosts whose immune reactivity had been suppressed. The response of the sensitized host was in all likelihood based predominantly on preformed humoral antibody; the effect of the immune interaction in this circumstance was the inhibition of vascular interconnection. The mechanism of inhibition of vascular connection by an antigen-antibody interaction remains to be elucidated; evidence in hand suggests that the immune interaction leads to a nonspecific inhibition of endothelial cell proliferation (20a).

Cellular and humoral components of the allograft reaction were to some extent separated in these experiments by the device of returning the allograft to its donor; they undoubtedly blend in allografts allowed to run their usual course to destruction. By the time humoral antibodies have formed in effective concentrations in a primary allograft reaction, the cellular response is likely to be well advanced. At this point it is difficult to assess the relative importance of cellular and humoral factors in graft destruction. The data from the returned allografts indicate that the cellular response in itself can produce epithelial cell destruction, and also that humoral antibodies, through an effect upon the vascular system, can produce graft destruction without cellular infiltration. Since there is evidence of increased endothelial cell turnover in skin allografts even after the initial vascular connections have been established (3), inhibition of endothelial proliferation may well be part of the final allograft-rejection process. Moreover, even in a whole-organ graft, in which vascular connection is established at once by suture, the development of antibody may produce vascular effects that contribute to graft dysfunction and rejection, while the presence of preformed circulating antibody may lead to immediate rejection by affecting endothelium and small blood vessels (21).
SUMMARY

In order to gain added insight into the mechanisms of allograft destruction, skin grafts were returned to their original donors after remaining as allografts long enough to induce immunity in the intermediate host but not long enough to cause destruction of the graft.

Upon their return to unmodified donors, such grafts became revascularized and remained viable. An intense cellular infiltration was incited within the graft and its draining lymph node by the interaction between immunologically competent cells, some antigenically activated, that were transferred from the intermediate host with the graft, and those of the final host, the original donor. This immune interaction excited a nonspecific granulocytic and histiocytic response, which led to the destruction of the adjacent epithelium already re-accepted within its native habitat. This mechanism of epithelial destruction required vascular connection to permit the cellular infiltration, and was unlikely to have primarily involved circulating antibody.

When similar grafts were returned to donors that had been sensitized to the intermediate host, vascularization and reacceptance of the graft did not occur. No cellular infiltration took place in the graft and no lymph node response was evoked. The returned grafts were cast off as full-thickness sloughs. Here the mechanism of graft rejection was apparently an interaction between the preformed antibody of the specifically sensitized host and the allogeneic cells transferred from the intermediate host; this interaction prevented the vascularization of the graft, even though the endothelia involved were autologous.

In unmodified allografts, both the character and the variability of the histologic patterns can be accounted for by the superimposition, in differing rates and degrees, of humoral vascular effects upon cellular events already in progress.

REFERENCES

1. Gutmann, R. D., M. F. Kraus, and M. F. Dolan. 1964. Rejection of isogeneic murine skin grafts following exposure to allogeneic ribonucleic acid. Nature (London). 203:196.
2. Burrows, L., H. Muir, and J. F. Mowbray. 1966. Rejection of mouse autografts with a purified allogeneic RNA. Ann. N. Y. Acad. Sci. 129:250.
3. Lambert, P. B., and H. A. Frank. 1967. Local recognition of histocompatibility differences in skin grafts. Science (Washington). 155:99.
4. Svet-Moldavsky, G. J., J. Mkheidze, A. L. Lioznev, and H. Ph. Bykovsky. 1968. Skin heterogenizing viruses. Nature (London). 217:102.
5. Hellman, K., and D. I. Duke. 1967. In vitro alteration of skin graft antigenicity. Transplantation. 5:184.
6. Medawar, P. B. 1944. The behaviour and fate of skin autografts and skin homografts in rabbits. J. Anat. 78:176.
COMPONENTS OF THE ALLOGRAFT REACTION

7. Edgerton, M. T., and P. J. Edgerton. 1957. Studies on reversibility of homograft rejection. Surg. Forum. 8:593.
8. Silvers, W. K., R. E. Billingham, and B. H. Sanford. 1968. The H-Y transplantation antigen: a Y linked or sex influenced factor? Nature (London). 220:401.
9. Steinmuller, D. 1967. Immunization with skin isografts taken from tolerant mice. Science (Washington). 158:127.
10. Brautbar C., D. Nelken, and J. H. Boss. 1969. Autograft rejection: effect of prior transplantation to allogeneic and xenogeneic hosts. Transplantation. 8:121.
11. Dempster, W. J. 1968. Letter in Lancet. 1:145.
12. Murray, J. 1963. In the discussion section. Ann. Surg. 158:641.
13. Clark, D., J. E. Fokker, R. A. Good, and R. L. Varco. 1968. Humoral factors in canine renal allograft rejection. Lancet. 1:8.
14. Armstrong, M. Y. K., R. S. Schwartz, and L. Beldotti. 1967. Neoplastic sequelae of allogeneic disease. Transplantation. 6:1380.
15. Brent, L., J. B. Brown, and P. B. Medawar. 1959. Skin transplantation immunity in relation to hypersensitivity reactions of the delayed type. In Biological Problems of Grafting. Charles C Thomas, Publisher, Springfield, Ill. 65.
16. Dvorak, H. F., T. U. Kosunen, and B. H. Waksman. 1963. The “transfer reaction” in the rabbit. Lab. Invest. 12:58.
17. Elkins, W. L., and R. D. Gutmann. 1968. Pathogenesis of a local graft versus host reaction: immunogenicity of circulating host lymphocytes. Science (Washington). 159:1250.
18. Dumonde, D. C., R. A. Wolstencroft, G. S. Panayi, M. Matthew, J. Morley, and W. T. Howson. 1969. “Lymphokines”: non-antibody mediators of cellular immunity generated by lymphocyte activation. Nature (London). 224:38.
19. Billingham, R. E., and L. Brent. 1957. A simple method for inducing tolerance of skin homografts in mice. Transplant. Bull. 4:67.
20. Simonsen, M. 1962. Graft versus host reactions: their natural limitations and applicability. Progr. Allergy. 6:349.
20a. Lambert, P. B. 1970. The effect of immunity on the vascularization of skin allografts. Transplantation. In Press.
21. Jeannet, M., V. W. Pinn, M. H. Flax, H. J. Winn, and P. S. Russel. 1970. Humoral antibodies in renal allotransplantation in man. N. Engl. J. Med. 282:111.