THE VASCULAR BED AS THE PRIMARY TARGET IN THE DESTRUCTION OF SKIN GRAFTS BY ANTISERUM

I. Resistance of Freshly Placed Xenografts of Skin to Antiserum*

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We have shown that both xenografts and allografts of rat skin can be acutely and severely damaged by antisera specifically reactive with graft antigens, and that in appropriate circumstances the phenomenon can be elicited routinely (1, 2). A matter of paramount importance among the variables that influence the occurrence of this form of immunologically mediated tissue damage is the interval of time between the placement of the grafts and the administration of antiserum (3). Contrary to widely held views on the special vulnerability of grafts that are healing into place, it has been found that antiserum injected at the time of grafting or within 5–6 d thereafter has little detectable influence on the course or time of survival of the transplanted skin. This period of insusceptibility is rapidly succeeded by a state of sensitivity to humoral antibody that, in immunosuppressed hosts, reaches a peak of intensity at about 14–16 d after grafting and persists at decreasing levels for an additional 3 wk. Grafts that survive beyond that period of time regain their resistance to antiserum, and this state is maintained for the duration of survival of the grafts.

We have analyzed these changes in the responses of xenografts of skin to antisera, and we have found that the initial state of insensitivity differs substantively from that observed in long-standing grafts. We describe here the results of our studies on freshly placed grafts, and in a succeeding paper (4) we report on the mechanisms involved in the acquired resistance of grafts that have survived for relatively long periods of time.

Materials and Methods

Animals. B6AF$_1$ and CAF$_1$ mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Lewis (LE) and (Le × BN)F$_1$ hybrid rats (LBN) were obtained from Microbiological Associates, Walkersville, Md. CD rats were purchased from the Charles River Breeding Laboratories, Wilmington, Mass.

Antisera. Rabbit anti-mouse thymocyte serum (RAMTS)[1] was prepared as described (5) or was obtained from Microbiological Associates. 13 pools of antisera were used, the least potent of which extended the mean survival time of rat skin grafts to 32.5 ± 11 d. Rabbit anti-rat

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[1] *Abbreviations used in this paper:* MARS, mouse anti-rat serum; RAMTS, rabbit anti-mouse thymocyte serum; RARS, rabbit anti-rat serum.

serum (RARS) was prepared by injecting mixtures of rat thymus and lymph node cells into rabbits according to the schedule used for preparing RAMTS. Portions of this serum were absorbed with mouse cells to remove cross-reacting antibodies. Mouse anti-rat serum (MARS) was prepared in $B6AF_1$ and CAF_1 mice by injecting them at weekly intervals with 10^7–10^8 rat lymphoid cells per mouse. Mice were bled each week and a single large pool of antiserum was made. In some cases the rat cells were emulsified with Freund's complete adjuvant and injected intraperitoneally at weekly intervals until ascites developed. The ascites fluid was collected, pooled, and used in some experiments in place of MARS. There were no detectable differences in the biological activities of the serum and ascites fluid, and for convenience both are referred to as MARS.

Skin Grafts. Ear skin was grafted as described previously (5). Individual grafts measured 15–20 mm along the longest diameter and 14–15 mm along the shortest diameter. Grafts that were to be retransplanted to new recipients were excised from their primary hosts together with a cuff of surrounding host skin. Subcutaneous tissue was removed from the undersurface of the graft by carefully scraping with a No. 10 surgical blade. As the secondary recipients were syngeneic to the primary ones, it was unnecessary to remove the cuff of primary host tissue before regrafting. All grafts were anchored to their beds at several points through the use of collodion flexible (Merck Chemical Division, Merck and Co., Rahway, N. J.). This procedure was especially helpful when two grafts were placed simultaneously on the same recipient.

In those special cases in which dressings were not applied to the grafts, the skin was held in place by interrupted sutures of 6-0 silk thread. To prevent damage to these grafts, the toenails of the recipients were clipped.

Experimental System. Mice were thymectomized 2–3 wk before receiving rat skin grafts, and they were injected intraperitoneally with 0.25 ml of RAMTS 2 d before grafting, on the day of grafting, and on the 2nd and 4th d after placement of the grafts. At various times after transplantation they were injected with MARS and the effects of these injections on the grafts were studied.

Immunofluorescent Studies. Grafts were excised from their hosts and cryostat sections of them were prepared for studies with fluorescent antisera as described previously (6). Fluorescein-conjugated antibodies reactive with rabbit IgG, mouse IgG, mouse C3, chicken IgG, or mouse serum albumin were obtained from N. L. Cappel Laboratories (Cochranville, Pa.). In some experiments, the hosts had been injected with anti-graft serum before removal of the grafts, and in these cases sections of tissue were stained directly with fluoresceinated anti-immunoglobulin (Ig) or anti-C3 reagents. In other cases, sections of frozen skin were treated in vitro with MARS or rat anti-mouse serum and then with fluoresceinated anti-Ig serum of appropriate specificity.

Results

Sensitivity of Skin Grafts to MARS Administered at Various Intervals after Transplantation. Table I contains a summary of the results of a large number of experiments in which MARS was administered on the day of transplantation or at a single interval thereafter. As we have reported previously (2, 3), the effects of the antiserum depend largely on the time at which it is given relative to the time of grafting. MARS given to 114 graft recipients during the first 6 d after transplantation had no evident effect on the healing processes or on the time of survival of the grafts. However, the sensitivity of the grafts increased sharply over the next week, reaching a peak at 14–16 d, and then declined steadily over the next 3 wk. During the period of peak sensitivity, intense inflammatory responses characterized by edema, erythema, and local hemorrhage were induced in 181 of 194 grafts that were tested, and 171 of these grafts were destroyed as an immediate consequence of the antibody-mediated inflammation. The small number of grafts (13 of 194 tested) that were unaffected by antiserum administered at 14–16 d after grafting were retested 2–3 d later and all but 2 were susceptible to the second injection of MARS. There are thus striking and

TABLE I

Response of Rat Skin Grafts to MARS Administered at Various Intervals after Transplantation

Day of injection*	Number of mice	Inflammation‡	Graft destruction§
		%	%
0–6	114	—‖	0
8	10	—	30
9–12	29	90	72
13–16	194	93	88
17–25	46	89	67
26–36	40	58	10
38–66	47	0	0

* Each mouse received 0.5 ml of MARS intraperitoneally.

‡ Erythema, edema, and local hemorrhage.

§ These figures indicate the percentage of cases in which graft destruction was an immediate consequence of the inflammation induced by antiserum.

‖ These grafts were covered by dressings at the time of testing and were not observed for changes during the period immediately after the administration of antiserum.

unexpected differences between the reactions of freshly placed and established xenografts of skin to the injection of anti-graft sera. These differences were observed consistently when a wide variety of pure strain and F_1 hybrid mice were used as recipients of rat skin grafts, and also when allografts of rat skin were used (2). The insensitivity of freshly grafted skin to antiserum seems, therefore, to be of widespread occurrence and the basis of such insensitivity, which is of potential interest from both practical and conceptual points of view, is analyzed in the following experiments.

Influence of Surgical Dressings and the Regimen of Immunosuppression on the Responses of Freshly Placed Rat Skin Grafts to MARS. We were initially concerned that the resistance of freshly placed grafts might turn on the unusual circumstances in which they were tested. At the time of challenge, these grafts were covered with protective dressings that were kept in place until the 8th d after grafting. It seemed possible that MARS did in fact cause inflammation in the graft beds but that the dressings protected the grafts from extensive damage and enabled them to heal, or in some cases to re-heal, into their beds. However, when grafts were sewn into place on the recipients, and the dressings were omitted, the period of early resistance was still observed. None of 52 such grafts that were challenged on days 0–8 after grafting were visibly affected, whereas all of 9 grafts challenged on day 14 with MARS were acutely destroyed. Furthermore, 12 grafts that had been in place for 14 d were rebandaged before challenge with MARS and all of them were fully susceptible to that agent.

The circumstances in which early grafts were challenged with MARS were unusual insofar as their responses may have been modified by the concurrent or proximate administration of the RAMTS used as a suppressive agent. However, that was not the case, as shown by the results of experiments in which RAMTS was given well in advance of grafting (12, 10, 8, and 6 d before transplantation). Grafts were placed on 33 mice, small groups of which were injected with MARS at 0, 3, 4, 6, 10, 14, or 16 d later. The development of sensitivity to antiserum in these grafts paralleled precisely that observed in grafts on control groups of mice that had received RAMTS according

to our regularly used schedule. None of 21 grafts tested through day 6 was sensitive; one of 4 tested on day 10, and all of 6 tested on days 14 or 16 were destroyed as a result of the administration of antiserum. Moreover, the same pattern of acquisition of sensitivity to MARS was observed for grafts placed on 20 BALB/c *nu/nu* mice that received no RAMTS.

Detection of Ig and C3 on the Vessels of New Grafts after the Injection of Their Hosts with Anti-Graft Sera. Our earlier studies had indicated that antiserum-mediated destruction of skin grafts is a consequence of an acute and intense vasculitis initiated by the reaction of antibodies with antigens on endothelial cells of the grafts. This view was strongly supported by the results of studies showing, through the use of immunofluorescent reagents, the presence of Ig and C3 on the luminal surface of graft vessels within minutes of the injection of MARS or RARS (7). We have looked, therefore, for the deposition of Ig and C3 in vessels, or other parts of freshly grafted skin, during the period when it is insensitive to MARS. Anti-graft serum, either MARS or RARS that had been absorbed with mouse tissues, was injected intraperitoneally at a dose of 0.5 ml, and the grafts were removed 2–4 h later and prepared for examination with fluoresceinated antisera as described elsewhere (6). Rabbit antiserum was used in some experiments because it was thought to facilitate the distinction between endogenous and injected immunoglobulins in this experimental system.

In practice there was little difference in the observations made with RARS and MARS, but it is clearer in the case of RARS that the Ig on the vessel walls comes from the injected material. In Table II, we present results of a study in which RARS was injected into mice bearing rat skin grafts that had been in place for 2–7 d.

None of five grafts that had been in place for 2 d at the time of administration of RARS contained vessels that were stained with fluoresceinated antisera, and in only one of three grafts that were removed on day 3 after grafting were stained vessels found. In that graft, only a small number of vessels were stained with anti-Ig (<10%) or anti-C3 (<20%). When RARS was injected on day 4, there was widespread and readily detectable staining of the luminal surface of vessels by both anti-Ig and anti-C3 in five of eight grafts tested. Beginning on day 5, all grafts tested had numerous

TABLE II

*Detection of Injected Ig and Endogenous C3 in the Vessels of Rat Skin Grafts during the 1st wk after Transplantation**

Day of injection	Number of mice	Number grafts with vessels stained by		
		Anti-rabbit Ig	Anti-mouse C3	Anti-mouse Ig
2	5	0	0	0
3	3	1‡	1‡	0
4	8	5	5	0
5	3	3	3	0
6	3	3	3	0
7	3	3	3	0

* Recipients were injected intraperitoneally on the days indicated with 0.5 ml of RARS that had been absorbed with mouse tissues. 4 h later, the grafts were removed and frozen sections of them were stained with fluoresceinated antibodies of the specificities indicated. Mouse skin from these animals were unstained by all of the reagents used.

‡ Staining of <10% of vessels with anti-Ig and <20% with anti-C3.

vessels that stained with both anti-rabbit Ig and anti-mouse C3, as shown in Fig. 1. In none of the cases in this entire series of experiments was there notable staining of vessels or other graft elements with anti-mouse Ig or anti-mouse albumin, although a diffuse blush in the edematous connective tissue was seen. Deposition of Ig and C3 on the luminal surfaces of graft vessels was again readily detected through the use of fluoresceinated antisera after injecting the graft bearers with MARS (Fig. 2).

The patterns of staining described here are fully consistent with numerous observations on the time of vascularization of free grafts of skin, *viz.*, that circulation is first detected on day 3–4 after grafting and is abundant thereafter. More important for the present study, these data show conclusively that Ig and complement components reach and react with endothelial cells of the grafts at a time when they are still completely resistant to anti-graft serum. Thus the resistance of the grafts appears to be related either to their transient insensitivity to the mediators developed by the immune reactions taking place within the graft vessels or to local depletion of such mediators.

Development of Sensitivity to Antiserum in Grafts Placed Simultaneously on the Same Recipient. Primarily vascularized grafts of rat hearts and rat kidneys placed in mice are sensitive to antisera immediately after transplantation (8) and remain so for at least 8 wk thereafter, which suggests that the resistance of freshly placed grafts of skin can

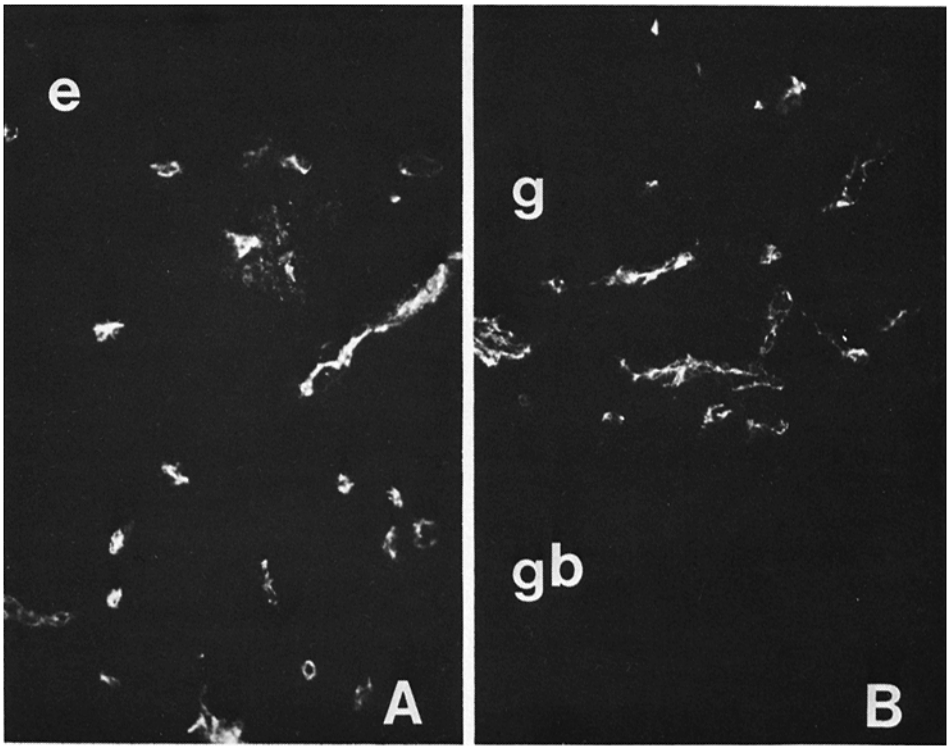

FIG. 1. Immunofluorescence photomicrographs of a rat skin xenograft 14 d after grafting onto an immunosuppressed mouse and 4 h after RARS was given intraperitoneally. The cryostat section, stained with fluorescein-conjugated anti-rabbit IgG, shows bright fluorescence along the endothelium of the microvasculature of the graft (A) but not in the connective tissue or epidermis (e). The vessels in the graft (g) but not in graft bed (gb) show bright staining (B).

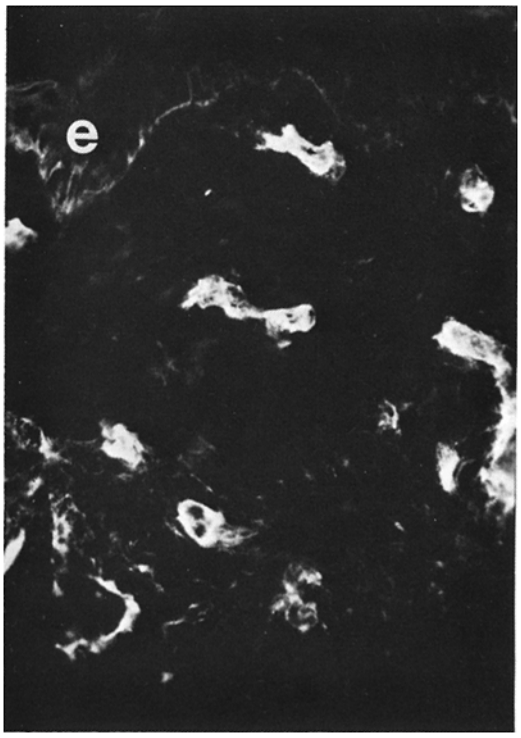

FIG. 2. 14-d rat skin xenograft from a B10.D2 old (C5 deficient) mouse given MARS intraperitoneally 3 h earlier and stained in vitro with fluorescein-conjugated anti-mouse C3. The endothelium of the graft vessels stains brightly. The epidermis (e) and connective tissue are negative.

be traced to factors acting locally within the grafts. This point was examined in the present study by placing two skin grafts simultaneously on the same recipient and testing their responses to antiserum at various intervals after grafting. A predominant systemic influence would be indicated if grafts on the same animal invariably acquired sensitivity to antiserum at the same time, whereas the importance of local conditions would be indicated if in some animals one graft developed sensitivity, while the other remained resistant to antiserum. The distinction may, of course, be blurred to some extent by the influence of the general physiological state of the recipient on locally acting factors.

Immunosuppressed $B6AF_1$ mice received two rat skin grafts placed on opposite flanks. 6–15 d later, these recipients received a single injection of MARS, and the grafts were examined periodically for signs of inflammation and tissue damage. The results of this experiment are summarized in Table III. Various degrees of inflammation were detected in all but one graft, but in many cases there were striking differences in the intensity of responses of grafts on the same mouse. These differences were observed in mice of all groups but they were more common among mice that received MARS on days 6, 7, or 8 after grafting. The differences are best illustrated by considering the incidences of graft destruction that occurred as an immediate consequence of the administration of antiserum. When recipients were injected with MARS on days 6–8, 7 of 25 mice that were tested had one graft that was destroyed,

TABLE III

*Development of Sensitivity of MARS in Skin Grafts Placed Simultaneously on the Same Recipients**

Day of injection of MARS‡	Number of mice	Both grafts destroyed	Neither destroyed	One graft destroyed
6	8	0	5	3
7	11	4	4	3
8	6	5	0	1
9–15	14	13	0	1

* Each mouse received two grafts placed simultaneously on the left and right flanks.

‡ MARS was injected intraperitoneally at doses ranging from 0.1 to 1.0 ml within each group. Inflammation occurred in all but one graft.

whereas the other survived (Table III). In mice tested on days 9–15, all but one graft was destroyed; in one mouse injected on day 14, both grafts showed intense inflammation, but one recovered completely.

These results show that the development of sensitivity to antiserum can proceed independently in each of two grafts placed simultaneously on one recipient; and, accordingly, they provide evidence for the influence of local factors on this process.

Reversibility of the Acquired Sensitivity of Grafts to Antisera after Their Transfer to New Hosts. It is clear from the experiments described above that freshly placed skin grafts may remain resistant to antisera even after they have become vascularized, and even though their vessel walls bind immunoglobulins and C3. Furthermore, it is clear that the transition from resistance to susceptibility to antisera involves local changes in the grafts. Are these changes related to the processes by which the grafts heal into place or are they in some way induced by the new hosts? In our attempts to resolve this issue, we have determined the extent to which a state of recently acquired sensitivity can be reversed by retransplanting skin to new immunosuppressed recipients. Rat skin was transplanted to immunosuppressed mice and allowed to remain in place for either 5 or 13 d. The grafts were then removed and transplanted to secondary immunosuppressed hosts of the same genotype as the initial recipients. These secondary recipients received a single injection of 0.5 ml of MARS on day 1, 6, 14, or 15. The results of this experiment are presented in Table IV. Grafts that had been in place on primary recipients for 13 d, and could therefore be confidently assumed to have developed a state of intense sensitivity to MARS, became completely resistant to this agent when grafted to new recipients; this state of resistance was maintained for at least 6 d. However, when retransplanted skin was tested at 14 d after grafting, it was completely susceptible to MARS, as were control grafts that had been in residence on a single host for 14 d. The development of susceptibility to MARS seems not to involve permanent loss or gain of any substance in the graft. Rather, the short-lived insensitivity of freshly placed grafts seems to be associated with processes involved in the healing of free grafts.

Effect of MARS Injected Intracutaneously into Rats. Further evidence that the resistance of freshly grafted skin to antisera is related to the healing process rather than to host-induced alterations comes from experiments in which MARS was injected intracutaneously into rats. Individual rats received injections of 0.05 ml of various concentrations of antiserum at several sites along the dorsal surface of the trunk, and these

TABLE IV

*Effect of MARS on Rat Skin That Had Been Transplanted to Secondary Recipients**

Day of injection‡	Fresh grafts§	Skin regrafted at 13 d (number destroyed)	Skin regrafted at 5 d (number tested)
1	0/4	0/8	0/4
6	0/2	0/6	0/4
14, 15	5/5	10/10	5/5

* Rat skin was transplanted to immunosuppressed $B6AF_1$ mice and at 5 or 13 d after grafting the skin was removed and placed on new immunosuppressed $B6AF_1$ mice.

‡ Day 0 is taken as time of secondary grafting. Each mouse received 0.5 ml of MARS intraperitoneally.

§ Control mice received fresh grafts only on day 0.

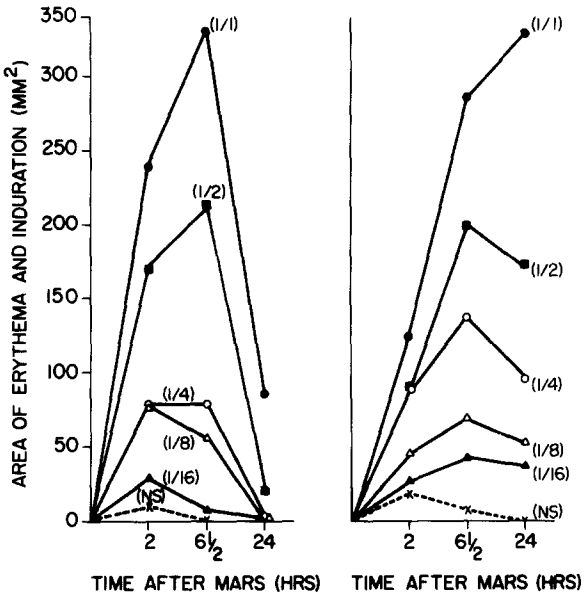

FIG. 3. The effect of MARS administered intracutaneously to rats. Multiple sites were injected with 0.05 ml of undiluted serum or a dilution thereof, up to 1:16. Normal serum (NS) was administered as a control. Data are presented for 2 of 10 rats that were treated.

sites were monitored for signs of inflammation and tissue damage. In all of 10 rats that were so treated, MARS caused intense inflammatory responses that proceeded to necrosis at the higher concentrations of serum. The courses of the reactions in typical recipient rats are presented in Fig. 3. The data shown in the figure were obtained by measuring two diameters of the lesions, which were circular, and using the average of these measurements to calculate the areas of erythema and induration.

Normal serum caused small amounts of edema that were measurable at 1–2 h but were almost completely resolved 6.5 h after injection. Sites of injection of MARS developed edema and erythema by 1–2 h and at 6.5 h there was necrosis that measured up to 0.5 × 0.5 cm. The lesions reached a peak of intensity at 4–8 h, and

resolved gradually over a period of 3–5 d. The evolution of these lesions followed a course very similar to that seen in skin grafts after treatment of their hosts with anti-graft serum. They were not typical Arthus reactions, however, as indicated by the fact that we were unable to detect in MARS any precipitating antibodies for rat serum or tissue extracts. Thus, skin is fully sensitive to antiserum when it is removed from the donor rats and on being transplanted it enters a state of resistance that persists for 5 or 6 d. This observation clearly supports the notion that the period of resistance occurring immediately after grafting is related to the processes by which free grafts heal into place and become vascularized.

Rechallenge on Day 14 of Skin Grafts That Had Been Found Resistant to MARS Immediately after Grafting. We have shown conclusively that anti-graft serum administered during the first 5–7 d after grafting has no detectable effect on the survival of grafts, although it had in many instances led to the deposition of Ig and C3 on the luminal surfaces of graft vessels. Moreover, these grafts remained intact even after they had reached an age at which they should have developed susceptibility. It was therefore of interest, especially in view of our observations on the replacement of donor endothelium by host cells in long-term skin grafts (8), to determine the susceptibility at 14 d of those grafts that had previously displayed resistance to MARS. We wanted to determine whether the early administration of antiserum had in any way damaged donor endothelium, leading to its replacement by host cells and accompanying resistance to antiserum.

As indicated in Table V, 22 mice whose grafts had been resistant to MARS injected on day 1, 4, 6, or 8 after transplantation were again injected with antiserum when the grafts were 14 d old. Intense inflammatory responses developed in all of these grafts within 1 or 2 h after administration of serum, and 21 of the 22 grafts were destroyed as a result of the inflammation. Thus, there is no indication that the interaction of antibody and complement with endothelial cell surfaces in freshly placed grafts causes irreparable damage to such cells.

Discussion

The resistance of freshly placed grafts of skin to antisera has in a sense been established for many years, for there are numerous reports that record the failure of antisera, administered at the time of transplantation, to influence the fate of grafted

TABLE V

*Effect of the Administration of MARS during the 1st wk after Grafting on the Later Sensitivity of the Grafts to Antiserum Given at 14 d after Grafting**

Day of 1st MARS	Graft destruction‡		Inflammation‡ (2nd MARS)§
	1st MARS	2nd MARS	
1–4	0/18	17/18	18/18
6–7	0/5	5/5	5/5
—	—	8/11	10/11

* $B6AF_1$ mice bearing Le skin grafts received 0.5 ml of MARS during the 1st wk after grafting and again at day 14.

‡ Number positive/number injected.

§ Grafts were covered by dressings at the time of the first injection of MARS. Accordingly, occurrence of inflammation could not be determined.

skin (9). Until recently, however, it was not appreciated that this state of resistance is transient, and that grafts that survive for sufficiently long periods of time become highly susceptible to antiserum. This gradual development of sensitivity of skin grafts to humoral antibody has been observed by us in large numbers of rats and mice of diverse genotypes and its occurrence has been confirmed by Gerlag et al. (10) in their studies with mice. It is a phenomenon that is surprising in part because of intuitive feelings that grafts that are healing into place should be more vulnerable to immunologic assault than are established grafts; and in part because other types of grafts, e.g., primarily vascularized grafts of hearts and kidneys, are fully sensitive to antisera from the moment that transplantation is technically completed. Grafts of tumor cells are also sensitive to antiserum immediately after they are placed in new hosts and indeed their sensitivity, which is at a peak at this point, is rapidly lost thereafter. Why then should grafts of skin be completely insensitive to humoral antibodies in experimental circumstances in which grafts of other kinds are so susceptible to these agents? The data that we present here show conclusively that the resistance of freshly grafted skin does not turn on the protective effects of the dressings that have been used, nor can it be attributed to the regimen of immunosuppression that was used in many of the experiments. Furthermore, a delay in the development of sensitivity occurs in the case of skin grafts, but not in the case of grafts of other kinds that are transplanted in similar circumstances, which points to the importance of factors operating locally rather than systemically. This conclusion is supported by the observation that when mice receive two skin grafts simultaneously, the grafts often develop sensitivity at different rates, and, as shown here, one graft may be severely damaged by antiserum, whereas a contralaterally placed graft is not noticeably affected. An explanation for the exceptional behavior of skin grafts must then be sought in local conditions that are peculiar to such grafts.

Free grafts of skin, unlike primarily vascularized grafts, do not have vascular connections with their hosts until about the 4th d after transplantation, a point that is affirmed by our studies with fluorescein-labeled antibodies. It would not be possible, therefore, to induce immediate vascular injury in recently placed grafts, and the induction of vascular injury seems to be a *sine qua non* of antiserum-mediated destruction of organized tissue grafts (4). This explains the resistance of grafts for the 1st 4 d after transplantation, but even after grafts are vascularized they remain resistant to antiserum for an additional 2 or 3 d and peak sensitivity is not acquired until about the 12th or 14th d after grafting. This is surprising in view of the fact that it can be shown that the luminal surfaces of the vessels of these grafts bind readily detectable amounts of injected antibody, which in turn bind detectable levels of endogenous C3. The resistance of grafts in these circumstances is clearly not attributable to the failure of primary effector substances to reach their targets. It is more likely that the target tissue fails to respond to the effector substances.

This state of indifference or nonresponsiveness to phlogistic substances is associated with the technical procedure of grafting, a conclusion based largely on two observations reported here. First, skin is sensitive to antiserum just before it is removed from the donor rats and again at about 7 or 8 d after transplantation. It is resistant only during the period immediately following grafting. Second, grafted skin that has developed a high degree of sensitivity to antiserum loses that sensitivity if it is regrafted, and then subsequently regains it. Evidently, the resistance of freshly grafted

skin to antiserum derives from the early effects of transplantation on vessels within the graft and the graft bed. These vessels are subjected to mechanical trauma and anoxia during the transplantation procedure and their recovery from such damage entails vascular regeneration as well as the formation of new vessels. It is known that newly formed and regenerating vessels of the microvasculature are structurally immature, and Hurley (11) has reported that the vessels in granulation tissue are unresponsive to vasoactive substances for several days or weeks after they are formed. He has suggested that this lack of responsiveness may be essential to the proper development of vessels, especially those that are forming in areas of inflammation that abound in vasoactive materials. In any event, it supplies an adequate explanation for the resistance of freshly placed grafts to antiserum. The young vessels in the graft and adjacent bed are unable to respond to the vasoactive materials released or activated by the reaction of antibodies with graft antigens, and because tissue damage occurs secondarily to vascular injury the graft is spared. This explanation is entirely consistent with the observation that primarily vascularized grafts of hearts and kidneys are sensitive to antisera immediately after transplantation, for in these grafts there is no need for regeneration of the microvasculature and hence no reason for transient resistance to vasoactive substances. It is also consistent with our observations that long-term skin grafts, in which graft endothelium has been replaced by host cells, become resistant to antisera, for in this circumstance antibodies specifically reactive with graft antigens can no longer combine with endothelial cells to trigger the vascular injury that underlies graft destruction. Hence, the oft-reported resistance of skin grafts to humoral antibodies is not, as previously thought, due to peculiar properties of skin *per se*, but to peculiar properties of newly formed and regenerating blood vessels that are essential to the healing process.

Endothelial cells are not the only cells that are subjected to anoxia and trauma in the grafting procedure, and it is possible that damage to other elements of skin grafts contributes to their transient resistance to antiserum. Disruption of mast cells, for example, may lead to temporary loss of sources of vasoactive materials that play a role in antibody-mediated damage to tissues. Such a process would act in a manner complementary to the postulated low state of responsiveness of regenerating vessels to inflammatory stimuli.

The sensitivity of freshly grafted tumor cells to humoral antibody is not inconsistent with these views on the resistance of skin grafts. As we have pointed out elsewhere (12), the mechanisms involved in the destruction of tumors by antiserum are highly effective against small numbers of targets, but their effectiveness is very noticeably diminished as the size of the tumor graft is increased. These mechanisms can scarcely be expected to influence the course of free grafts of skin or primarily vascularized grafts of hearts or kidneys. The vulnerability of tumors to antiserum decreases progressively after grafting, which may be due to the corresponding increase in the size of the tumor or to the isolation of the tumor by the vasculature supplied by the host. This and other aspects of the role of the vascular bed in antibody-mediated damage to tissues is considered in more detail in the following report (4).

Summary

Rat skin grafted onto immunosuppressed mice is resistant to mouse anti-rat serum during the first 7–10 d after transplantation. It gradually acquires susceptibility,

reaching a peak of sensitivity at 14–16 d after grafting. The grafts remain sensitive to antiserum, though at decreasing levels for an additional 3 wk, and grafts that persist beyond that time are resistant to antiserum for as long as they survive. In the study reported here, it is shown that the initial period of resistance to antiserum is due to factors acting locally within the graft and is entirely uninfluenced by the regimen of immunosuppression or the protective dressings that are used. After administration of antiserum, deposits of the injected immunoglobulin and of endogenous C3 are found on the luminal surfaces of graft vessels, although no significant tissue damage is observed.

Rat skin that has become highly sensitive to antiserum 14–16 d after transplantation loses that sensitivity if it is regrafted to a new recipient, and then regains it 8–10 d later. Thus, the resistance of freshly grafted skin to antisera is associated with the process of healing into place, a conclusion that is supported by the observation that the intracutaneous administration of antisera to rats causes intense local inflammation and necrosis. The skin is therefore sensitive just before it is removed for grafting, but temporarily loses sensitivity thereafter. Resistance to antiserum during the first 3 or 4 d after transplantation is probably attributable to the fact that at that time grafts are vascularized poorly if at all. The state of resistance extends for several days after vascularization of the graft takes place and is then only gradually lost, a phenomenon that seems to be associated with the resistance of newly formed and regenerating blood vessels to vasoactive substances. This view is in accord with and, indeed, supports the idea that the induction of vascular injury is an essential step in antisera-mediated damage to tissue grafts.

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References

1. Winn, H. J., C. A. Baldamus, S. V. Jooste, and P. S. Russell. 1973. Acute destruction by humoral antibody of rat skin grafted to mice. The role of complement and polymorphonuclear leukocytes. *J. Exp. Med.* **137:**893.
2. Jooste, S. V., and H. J. Winn. 1975. Acute destruction of rat skin grafts by alloantisera. *J. Immunol.* **114:**933.
3. Jooste, S. V., H. J. Winn, and P. S. Russell. 1973. Destruction of rat skin grafts by humoral antibody. *Transplant. Proc.* **5:**713.
4. Jooste, S. V., R. B. Colvin, and H. J. Winn. 1981. The vascular bed as the primary target in the destruction of skin grafts by antiserum. II. Loss of sensitivity to antiserum in long-term xenografts of skin. *J. Exp. Med.* **154:**1332.
5. Baldamus, C. A., I. F. C. McKenzie, H. J. Winn, and P. S. Russell. 1973. Acute destruction by humoral antibody of rat skin grafted to mice. *J. Immunol.* **110:**1532.
6. Colvin, R. B., R. A. Johnson, M. C. Mihm, Jr., and H. F. Dvorak. 1973. Role of the clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in delayed skin reactions in man. *J. Exp. Med.* **138:**686.
7. Jooste, S. V. 1973. The role of humoral antibody in the rejection of transplanted skin. Thesis. University of Pretoria, Pretoria, South Africa.
8. Burdick, J. F., P. S. Russell, and H. J. Winn. 1979. Sensitivity of long-standing xenografts of rat hearts to humoral antibodies. *J. Immunol.* **123:**1732.

9. Billingham, R. E., and W. K. Silvers. 1971. The immunobiology of transplantation. Prentice-Hall Inc., Englewood Cliffs, N. J. 1.
10. Gerlag, P. G. G., R. A. P. Koene, J. F. H. M. Hagemann, and P. G. A. B. Wijdeveld. 1975. Hyperacute rejection of skin allografts in the mouse. Sensitivity of ingrowing skin grafts to the action of alloantibody and complement. *Transplantation (Baltimore)*. **20:**308.
11. Hurley, J. V. 1972. Acute Inflammation. The Williams and Wilkins Co., Baltimore. 68.
12. Winn, H. J. 1960. Immune reactions in homotransplantation. I. The role of serum antibody and complement in the neutralization of lymphoma cells. *J. Immunol.* **84:**530.