MAINTENANCE OF SKIN XENOGRAFTS OF WIDELY DIVERGENT PHYLOGENETIC ORIGIN ON CONGENITALLY ATHYMIC (NUDE) MICE*

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Congenitally athymic (nude) mice have been shown to accept skin allografts permanently (1–4). It has been further established that these mice accept skin grafts from several other species of rodents and lagomorphs, including rats, hamsters, and rabbits (5, 6). We have recently reported that nude mice will maintain for their lifetime full thickness grafts of normal human skin (7). This acceptance of human skin prompted us to attempt xenografts of ever increasing phylogenetic disparity in order to determine whether these athymic mice possess any ability whatsoever to reject foreign skin. We report here that nude mice maintain indefinitely intact skin grafts not only from distantly related mammals (cat, human), but from birds (chicken) as well. They also fail to reject skin grafts from reptiles (fence lizard and chameleon) and from amphibians (tree frog), although such grafts undergo certain morphological changes.

Materials and Methods

Mice.—Congenitally athymic mice, hereafter designated nude, were selected from a stock which has been backcrossed into the BALB/c strain. Nude mice and their phenotypically normal littermates were maintained on sterilized Purina 5010C feed (Ralston Purina Co., Inc., St. Louis, Mo.) and acidified-chlorinated water.

Skin Grafting.—Skin grafting was performed on mice of both sexes between 5–7 wk of age. Human skin was obtained from the foreskins of circumcised infants; cat skin specimens were taken from the ear, paw, and facial regions. Chicken skin grafts were prepared primarily from the cervical apterium (featherless skin) and its borders. A select few chicken grafts were prepared from the capital pteryla (contour feather tract) to include a maximal number of feathers or follicles; the feathers were plucked or trimmed 2 days before sacrifice for grafting. Skin from the large-scaled lizards (fence lizards, genus *Sceloporus*) was taken from the throat or abdominal regions, whereas that from the small-scaled lizards (chameleon, genus *Anolis*) and tree frogs (genus *Hyla*) was taken from any area of the trunk. All donor skins were prepared by pinning the entire specimen on a flat surface and gently scraping away all subcutaneous fascia. Circular grafts 1 cm in diameter were then cut with a carefully sharpened, sterile cork borer. The remainder of the grafting procedure was essentially that of Billingham (8). Prolonged graft protection was achieved by bandaging with trimmed Band-Aid Sheer Strips (Johnson and Johnson, New Brunswick, N. J.)

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The day of skin grafting was designated day 0. Graft success was judged by both outward graft appearance and histological examination of selected grafts. Grafts to be examined histologically were removed in their entirety, along with the surrounding mouse skin and underlying musculature, and fixed in 10% formalin. The specimens were then embedded in paraplast, sectioned, and stained with hematoxylin-eosin.

Thymus Gland Implantation.—Selected nude mice were each implanted in the axillary region with the thymus glands from two neonatal BALB/c donors.

RESULTS

Phenotypically normal, thymus-bearing littermates of nude mice, grafted as controls, uniformly rejected xenografts in a very predictable way. All grafts healed in by day 5 or 6 (with no apparent signs of immediate physiological incompatibility even in the case of reptile or amphibian skin), became inflamed and indurated by day 7–9, and rejected completely by day 8–12. Rejection time within this interval did not appear to be related to phylogenetic disparity between mice and the donor species.

Nude mice, in contrast, were shown never to reject in typical immunological fashion skin xenografts from any of the mammals, birds, reptiles, or amphibians used as donors (Table I). The precautions necessary to ensure graft survival, however, varied considerably with the origin of the donor skin. In general, we found that xenografts, unlike allografts, required prolonged (3–5 wk) protective bandaging in order to guarantee success of the graft. Failure to thus protect any xenograft very frequently resulted in graft failure as a result of mechanical injury due to scratching and biting by the recipient. Encumbrance of the initial plaster body cast was shown to cause substantial weight loss and decline in vigor among nude animals. For this reason, we uncast all mice as early as possible (day 5) and carefully (under sodium pentabarbital anesthesia)

TABLE I
Survival Times of Skin Xenografts from Donors of Four Taxonomic Classes on Nude Mice

Skin donor	Taxonomic class	Selected no. of grafts	Survival times
			*days**
Cat	Mammalia	8	61, 58, 104, 102
			62, 58, 56, 92
Human	Mammalia	10	60, 65, 57, 55, 81
			53, 55, 76, 80, 103
Chicken	Aves	10	55, 49, 50,‡ 70,‡ 47
			82,§ 82,§ 63,§ 63,§ 63,§
Fence lizard	Reptilia	8	22,‡ 28,‡ 41,‡ 34
			41, 34, 85,§ 68§
Chameleon	Reptilia	6	41,‡ 52, 70
			56, 51, 67§
Tree frog	Amphibia	3	16,‡ 40,‡ 73

* Determined by longevity of the grafted animals; rejection was not evident in any nude animal. All xenografts on phenotypically normal animals were completely rejected in 12 days or less.

‡ Sacrificed for histological sectioning.

§ Animal still alive with graft in place.

bandaged them with flexible, trimmed Band-Aids, leaving the *tulle gras* in place and exercising utmost caution to avoid disturbing the grafts. On day 8, the *tulle gras* was removed, and a new Band-Aid was applied. Thereafter graft examination was made by lifting the Band-Aid slightly each day.

Human skin grafts protected in this fashion always appeared healthy when uncast and maintained an excellent appearance until death of the recipient (Fig. 1). The histological appearance of a 26 day human skin graft can be seen in Fig. 2. Cat grafts, on the other hand, consistently appeared healthy at days 5–10, but then shed a dry scab-like outer "ghost" which contained all of the hair stubble on the graft. Generally the thicker the skin grafted, the thicker were the developing ghosts, being thickest with heavy facial skin grafts. These ghosts separated completely after 5–20 days, mimicking immunological rejection closely. Progressive shrinkage of the ghost from the graft periphery, however, revealed underlying viable, hair-producing cat skin, resulting eventually in a luxuriant tuft of cat fur which continued to grow until death of the recipient (Fig. 1).

Chicken grafts were considerably more difficult to establish. Our original grafting procedure entailed uncasting at day 7 with no further bandaging. In our initial studies, at least 20 nude mice grafted in this way all sloughed their grafts by day 20, leading us to the false conclusion that "rejection" had occurred (6). Close observation, however, revealed that the precipitous decline in graft health and subsequent inflammatory response were always preceeded by scratching and/or biting of the graft by the recipient. Prevention of this type of trauma by prolonged protective bandaging alleviated the "rejection" problem entirely, resulting in uniform acceptance of chicken skin grafts for the lifetime of the recipients (Table I). The outward appearance of a 32 day graft bearing feathers and the histological appearance of a 50 day featherless graft can be seen in Figs. 1 and 2, respectively. The smooth featherless skin of the cervical apterium was readily "accepted" upon being licked clean by the recipient after unbandaging and so could often safely be left unprotected after 3–4 wk. Follicle lumps in skin from the capital pteryla plucked free of feathers or feathers themselves, on the other hand, provided an apparent irritation to the recipient when unbandaged, and such skin grafts were quickly attacked by the recipients when left unprotected. For this reason, grafts containing feathers or feather follicles required constant bandaging. *De novo* feather eruption in follicle-bearing skin grafts was an unusual phenomenon, apparently because of feather ingrowth caused by pressure from the protective bandages.

Grafts of lizard skin also required extended protection. All lizard grafts on nude mice by day 12–18 shed a paper-thin ghost containing the scales present on the graft when transplanted. Although the scales were not replaced, the scale pattern remained evident at all times (Fig. 1). Histological examination of lizard grafts revealed an overgrowth of mouse epidermal cells above the transplanted lizard skin (Fig. 2). The similarity of histological architecture in grafted lizard skin to that of normal lizard skin (with scales removed) shown in Fig. 2 suggests that grafted reptile skin may at times retain at least part of its integrity despite overgrowth by nude mouse epidermis. In striking contrast, littermate controls always shed the entire lizard graft as a single scab-like unit. Grafts of tree frog skin likewise remained quite evident in outline and color for the lifetime of the grafted animal (Fig. 1). Histological examination showed, however, that the frog skin was overgrown by mouse epidermis in a manner similar to that seen in reptile grafts. A comparison of the histological architecture of normal tree frog skin with that of a tree frog skin graft (Fig. 2) also revealed a considerable degree of grafted skin disorganization. The extent of disorganization among the reptile and amphibian grafts examined was somewhat variable but was sufficient in some cases (tree frog,

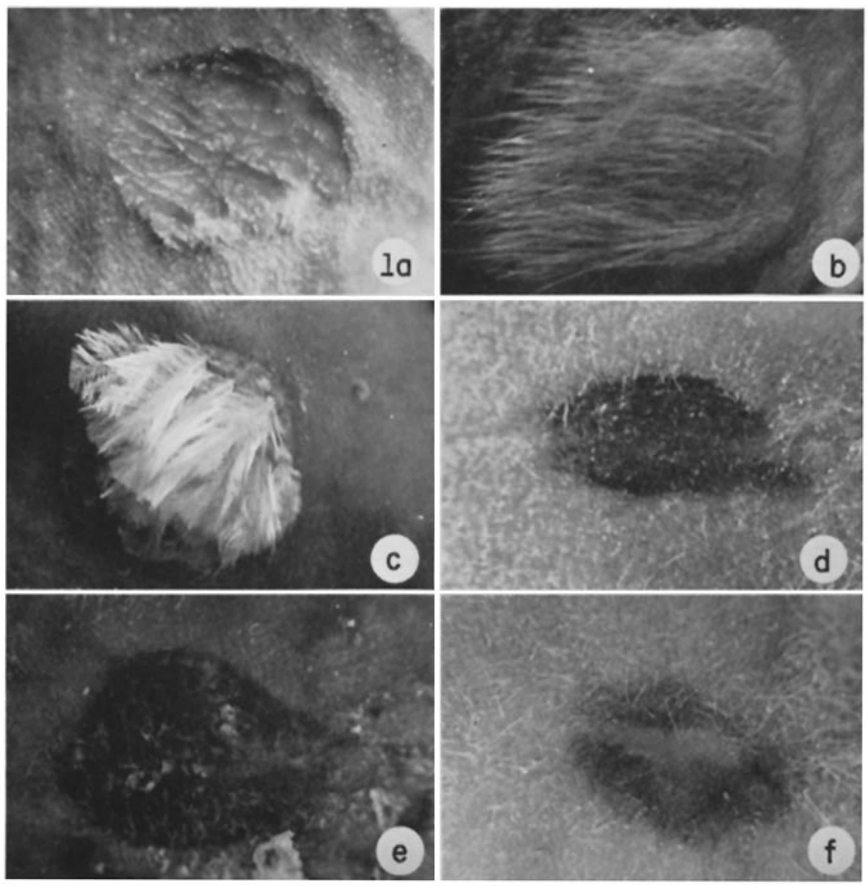

FIG. 1. Outward appearance of skin xenografts maintained on nude mice. (*a*) Human graft at day 60. (*b*) Cat graft at day 51; this graft began hair growth at 20 days and continued growth until death of the recipient at 102 days. (*c*) Chicken graft at day 32; this skin was grafted with feathers intact. (*d*) Chameleon graft at day 41. (*e*) Fence lizard graft at day 28. (*f*) Tree frog graft at day 40; the pale central area represents a white stripe on the living frog. Magnification, × 3.

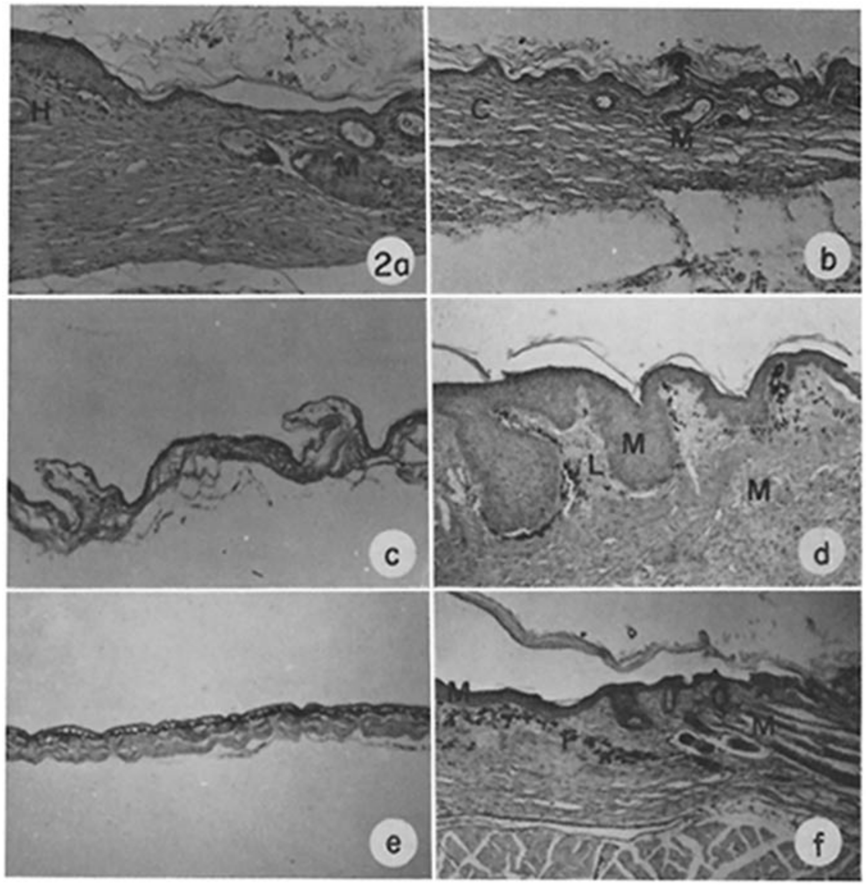

FIG. 2. Histological appearance of skin xenografts maintained on nude mice (*a*, *b* × 150; *c–f* × 75). Tissues in grafts are designated as mouse (M), human (H), chicken (C), fence lizard (L), or tree frog (F). (*a*) Human skin graft at day 26. (*b*) Chicken skin graft without feathers at day 50. (*c*) Ungrafted normal fence lizard skin, scales removed. (*d*) Fence lizard graft at day 22. (*e*) Ungrafted normal tree frog skin. (*f*) Tree frog graft at day 16. Note the overgrowth of the lizard and tree frog grafts by mouse epidermis (*d*, *f*). Such overgrowth was not apparent in the case of human or chicken grafts (*a*, *b*).

Fig. 2) to suggest that at times little more than some form of pigment retention remained of the original skin structure.

Table II presents the results of an experiment using thymus-implanted nudes which had received or were to receive skin xenografts; all nude mice receiving thymus implants rejected their foreign skin grafts. Two mice (no. 1 and 2) which had 35-day chicken grafts in place at the time of thymus implantation rejected on days 65 and 69, respectively, i.e., 30 and 34 days after receiving thymus glands. Six other mice (no. 3–8) which had thymus glands implanted from 6 to 63 days before receiving chicken skin grafts rejected those grafts in from 12 to 26 days. In this experiment, all nude mice without thymus implants retained their skin grafts without signs of rejection. Similarly, five mice (no. 9–13) with established thymic implants rejected human skin grafts in from 12 to 28 days; again, all nudes lacking implants retained their skin transplants without signs of rejection.

TABLE II

*Rejection of Skin Xenografts in Nude Mice Bearing Thymus Gland Implants**

Mouse no.	Species of donor skin	Day of thymus gland implantation‡	Day of rejection‡, §
1	Chicken	+35	+65
2	Chicken	+35	+69
3	Chicken	−63	+17
4	Chicken	−29	+12
5	Chicken	−29	+19
6	Chicken	−24	+26
7	Chicken	−6	+26
8	Chicken	−6	+26
9	Human	−38	+12
10	Human	−38	+13
11	Human	−20	+18
12	Human	−13	+27
13	Human	−13	+28

* Thymus glands from two neonatal BALB/c mice were implanted in the axillary region of each nude mouse.

‡ Day of skin grafting = day 0.

§ All nude mice lacking thymus implants failed to reject their skin transplants.

DISCUSSION

The data presented here demonstrate that nude mice are apparently incapable of immunologically rejecting xenografts, even from such phylogenetically distant forms as birds, reptiles, and amphibians. The principal consideration in ensuring success of such foreign grafts was found to be adequate protection from chewing or scratching disruption by the recipients. We saw no evidence of physiological incompatibility (9) between the skin of mice and that of distantly related forms which was of sufficient magnitude to mimic acute immunological rejection. This does not rule out the possibility that physiological incompatibility may have contributed to the disorganization of transplanted skin observed in the case of reptile and amphibian grafts.

Rejection of xenografts after thymus implantation into nude mice clearly establishes the definitive relationship of the thymic defect in these animals to their inability to reject such foreign skin. It has been suggested that in thymus-bearing normal mice, humoral antibodies may play some role in graft rejection, particularly in the case of xenografts (10–13). It has also been reported that humoral antibodies, in concert with complement and polymorphonuclear leukocytes, can cause xenograft rejection in thymectomized, antilymphocyte serum suppressed mice bearing rat skin grafts (14, 15). Our data support the conclusion either that primary xenograft rejection in mice is principally a function of thymus-mediated cellular immunity or that whatever humoral antibodies may be involved are formed in response to thymus-dependent antigens.

SUMMARY

Congenitally athymic (nude) mice accepted for their lifetime intact skin grafts from distantly related mammals (cat, human) and birds (chicken). They also failed to immunologically reject skin grafts from reptiles (lizards) and amphibians (tree frog), although the skin in these grafts underwent varying degrees of disorganization. A definitive role for the thymic defect in this failure to reject xenografts was established by showing that thymus implantation into nude mice enabled them to reject such foreign skin.

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