Long-Term Maintenance of Normal Human Skin on Congenitally Athymic (Nude) Mice[1] (37318)

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Congenitally athymic (nude) mice have been shown to accept allogeneic skin grafts permanently (1, 2). It has also been established that these mice accept skin grafts from rats, hamsters, and rabbits, with no signs of rejection during the life span of the recipients (3, 4). Further, it has been observed that primary malignant human tumors can be grown serially in nude mice (5, 6). Although cultured human malignant cells have been propagated in these mice, it has not yet been possible to similarly propagate normal, diploid human embryonic cells from culture (7). The latter studies would suggest that in transplants from a donor species as phylogenetically distant from the mouse as the human, graft survival might involve some degree of maligant invasiveness of the transplanted tissue. This supposition is consistent with the generalization that malignant grafts have a survival advantage over nonmalignant grafts of similar genetic origin (8). That there is no such requirement for malignant invasiveness to insure survival of tissues grafted across the wide human–mouse phylogenetic gap is established by our present communication. We report here the successful maintenance of full thickness grafts of human skin on congenitally athymic (nude) mice for the life-span of the recipients.

Materials and Methods. Mice. Congenitally athymic mice, hereafter designated nude, were selected from a stock which has been backcrossed into the BALB/c strain. All mice were maintained on sterilized Purina 5010C feed and acidified-chlorinated water. Grafting was accomplished on animals of both sexes between 5 and 8 wk of age.

Grafting technique. Normal human skin was obtained from the foreskins of circumcised infants and prepared by pinning the entire tissue 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 (9). Graft success was judged both by gross outward appearance and histological examination of grafts from selected individuals. 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. A very limited number of grafts were mechanically unsuccessful, primarily due to the graft slipping partially off the graft bed during casting. In such cases, the grafts appeared inflamed or partially necrotic upon removal of the protective casts, never healed in properly, and were completely shed within 6 to 10 days. These grafts were designated as technical failures and were not given further consideration.

Results. Generally a single phenotypically normal littermate control mouse was grafted with each skin specimen. These animals all showed a typical, rapid xenograft rejection pattern; healing in was accomplished by Day

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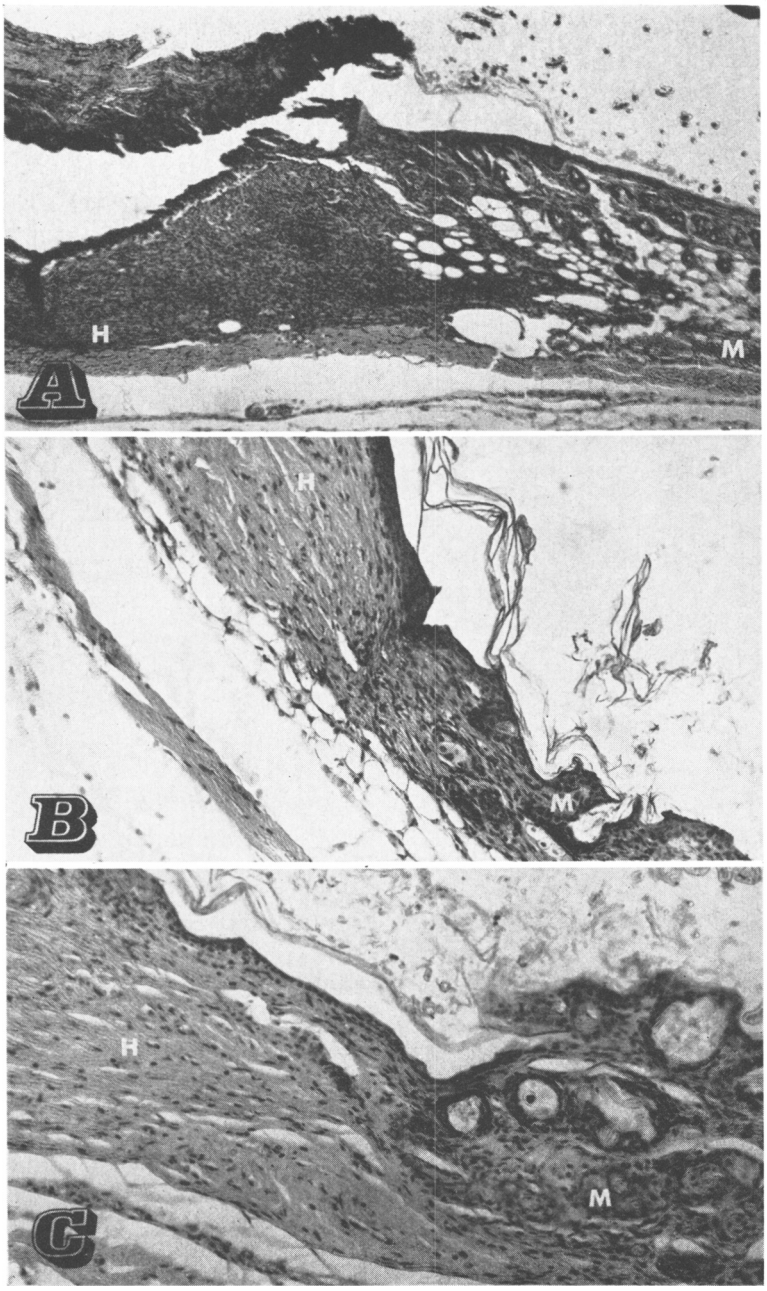

FIG. 2. Histological appearance of human skin grafts on mice (×200). Human skin (H) on left, mouse skin (M) on right. (A) Short-term (8 day) phenotypically normal mouse graft bed showing inflammatory cell infiltration. Rejection was in progress when the mouse was sacrificed. (B) Short-term (8 day) nude mouse graft bed showing healthy graft appearance. (C) Long-term (25 day) nude mouse graft bed showing continued absence of signs of graft rejection.

a variety of materials for carcinogenic effects on human skin without endangering a human subject. Such studies are in progress in our laboratory. The studies reported here clearly eliminate malignant invasiveness as a requirement for human graft acceptance by

nude mice. The possibility arises, therefore, that with appropriate transplantation technique, nude mice might also be useful for the *in vivo* study of other normal human tissues outside human subjects.

Summary. Congenitally athymic (nude) mice have been shown to accept for their lifetime full thickness, normal human skin grafts without immunosuppressive therapy. The biological and medical significance of the long-term maintenance of human tissues in nonhuman subjects is discussed.

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1. Wortis, H. H., Clin. Exp. Immunol. **8**, 305 (1971).

2. Pantelouris, E. M., Immunology **20**, 247 (1971).

3. Rygaard, J., Acta Path. Microbiol. Scand. **77**, 761 (1969).

4. Shaffer, C. F., Reed, N. D., and Jutila, J. W., Transplant. Proc. **5**, 711 (1973).

5. Rygaard, J., and Povlsen, C. O., Acta Path. Microbiol. Scand. **77**, 758 (1969).

6. Povlsen, C. O., and Rygaard, J., Acta Path. Microbiol. Scand. Sect. A **79**, 159 (1971).

7. Giovanella, B. C., Yim, S. O., Stehlin, J. S., and Williams, L. J., Jr., J. Nat. Cancer Inst. **48**, 1531 (1972).

8. Billingham, R., and Silvers, W., "The Immunobiology of Transplantation," 149 pp. Prentice-Hall, Englewood Cliffs, NJ (1971).

9. Billingham, R. E., *in* "Transplantation of Tissues and Cells" (R. E. Billingham and W. K. Silvers, eds.), p. 1. Wistar Inst. Press, Philadelphia (1961).

10. Lance, E. M., and Medawar, P. B., Lancet **1**, 1174 (1968).

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