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PROLONGED SURVIVAL OF HUMAN SKIN XENOGRAFTS ON ANTITHYMOCYTE SERUM-TREATED MICE: FAILURE TO PRODUCE VERRUCAE BY INOCULATION WITH EXTRACTS OF HUMAN WARTS*

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ABSTRACT

This report describes an experimental system whereby human skin was grafted to immunosuppressed mice, thus avoiding the restrictions imposed by the use of human subjects or tissue cultures. Grafts remained in good condition for the subsequent life of the animal, as long as eight months. The human skin xenografts were inoculated with an extract of verrucae vulgaris, but no warts developed during the period of observation.

Human epidermis is readily accessible for experimental studies but ethical considerations often limit in vivo experimentation. Tissue culture methods usually permit only very short-term investigations. We have attempted to develop an experimental system whereby human skin could be grafted to an immunosuppressed experimental animal and manipulated without any of the restrictions imposed by the use of human subjects or tissue cultures. We have previously reported long-term survival of human skin placed on an exteriorized hamster cheekpouch [1]. Approximately 50 percent of these human skin grafts survived for at least 14 weeks.

Although the human skin grafts were inoculated *with an extract of verrucae, no papillomas* developed in the grafts during 14 weeks of observation. However, when rabbit skin was grafted and infected with the Shope papilloma virus, almost all grafts developed papillomas. The failure of human skin to undergo papillomatous transformation was surprising. This failure might have been the result of a lack of viral infectivity or an insufficiently long period of observation. Infectivity of human wart virus can only be assessed in man [2] and not in tissue culture [3]. In view of the unknown oncogenic potential of this virus for the induction of neoplasms other than verrucae, human inoculation is not feasible. A longer period of observation of these human skin grafts was therefore important.

The purpose of these experiments was to maximally prolong the survival of human skin grafts placed on mice which were chronically immunosuppressed with antithymocyte serum (ATS). Rather than the hamsters used in the previous study, mice were employed as hosts because of the relatively smaller volumes of ATS required and subsequent reduced costs [4].

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MATERIALS AND METHODS

Skin grafting of mice. C57BL/6J male mice 4–5 weeks old were purchased from the Jackson Laboratory, Bar Harbor, Maine. Human skin grafts were prepared from radical mastectomy specimens as previously described [1]. Freshly excised human skin, 4 × 5 cm, was fastened with pushpins at 4 corners to a corkboard. The skin was washed with 70% ethanol and rinsed with ether. A sterile scalpel held parallel to the skin surface was used to slice off the split-thickness (0.5 mm) skin grafts. These grafts were collected and held in a Petri dish filled with Minimum Essential Medium (Eagle's). The grafts were trimmed with a scalpel to a square of 6–10 mm on an edge.

To prepare a graft bed in mice, the animals were anesthetized with Nembutal and the fur was clipped. The skin was washed with 70% ethanol and blotted dry with a sterile gauze pad. With curved scissors and a toothed forceps, the skin was elevated and snipped off to *create a defect which closely fit the graft. The underlying* panniculus carnosus and its rich blood supply were carefully preserved. The graft was gently pressed into the bed and excess fluid blotted off with sterile gauze. Dressings consisted of a 1-cm square of 2 thicknesses of Adaptic gauze (Johnson & Johnson, New Brunswick, N. J.) *and a covering of 2 wrappings of Dermicel First Aid* Tape (Johnson & Johnson) around the thorax. One week after grafting, the dressings were removed and the grafts inspected.

Antilymphocyte serum. ATS was purchased from Microbiological Associates, Bethesda, Md. This ATS was *previously assayed by the manufacturer and produced a* doubling of mean survival time of skin allografts across the H-2 locus in mice. We administered the ATS at a dosage of 0.25 ml (intraperitoneal) given on day 0, +1, +3, +5, +7, and three times each week thereafter. After inoculation with wart extract, the ATS injections were *reduced to two times per week until the death of the* animal. All animals were necropsied and tissues, including grafts, studied microscopically.

Preparation of wart extracts and inoculation of mice. Parings were obtained from two untreated plantar warts. Wart (2.0 gm) tissue was minced in 2.0 ml phosphate *buffered saline (PBS), pH 7.4, and homogenized in a* glass and teflon apparatus at 4° C. Particulate material was pelleted by low-speed centrifugation. 50 μl of supernatant solution were immediately inoculated into the xenografts of nine mice and the grafts were escarified

with a 30-gauge needle. Mice were hand-held until the inoculum dried. Four mice were inoculated at six weeks following grafting, and five mice were inoculated three weeks following grafting. After inoculation, the remaining wart extract was centrifuged at 174,000 × *g* for 60 min, and the pellet was resuspended in 0.1 ml PBS. This material was examined for the presence of virus in the electron microscope after negative staining with 1% phosphotungstic acid [5].

RESULTS

Fourteen mice received human split-thickness skin grafts. In one group of six mice, the grafts were 6–7 mm in diameter, and all the grafts survived. A second group of eight mice received larger grafts 8–10 mm in diameter, and in four of these animals the grafts were sloughed or rejected within one week. The failure of these grafts to "take" could be the result of mechanical detachment or immunologic rejection. Since the dressings were not removed until the seventh day, no prior observations were made and the question cannot be resolved. By three weeks all viable xenografts were reduced in size to approximately 4 mm (Fig. 1), and the 4-mm grafts survived without further size reduction until the death of the mouse. Two mice with intact xenografts succumbed within 30 days, but the other eight mice lived from three months to over eight months (Fig. 2).

Nine mice were inoculated with a freshly prepared extract of wart tissue and observed until ill or near death, at which time the animals were

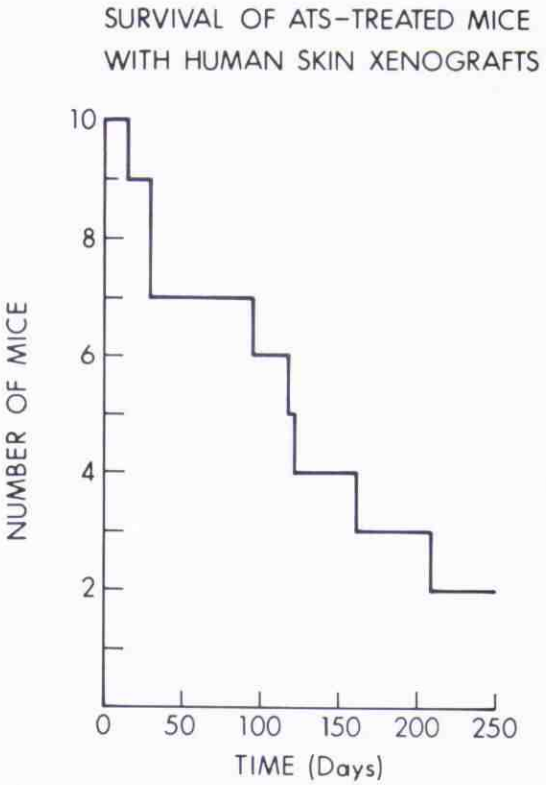

FIG. 2: Survival of human skin grafts in ATS-treated mice. All grafts were lost as a consequence of the death of the host.

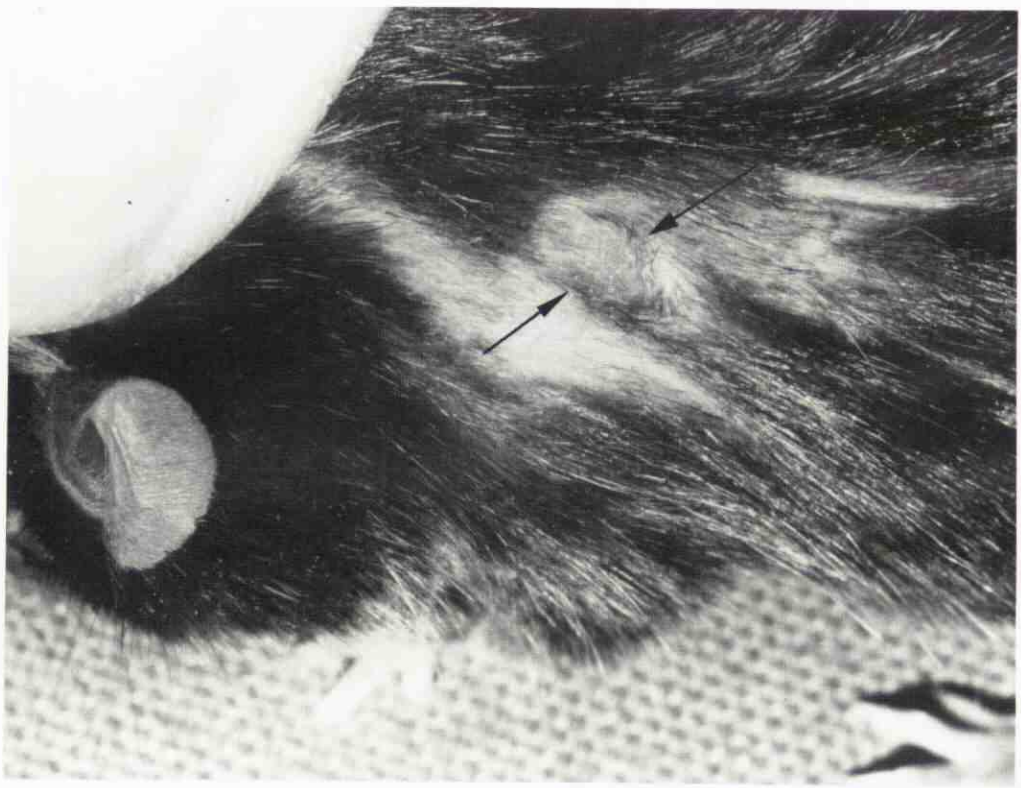

FIG. 1: Intact human skin graft present on ATS-treated mouse for eight months (between arrows).

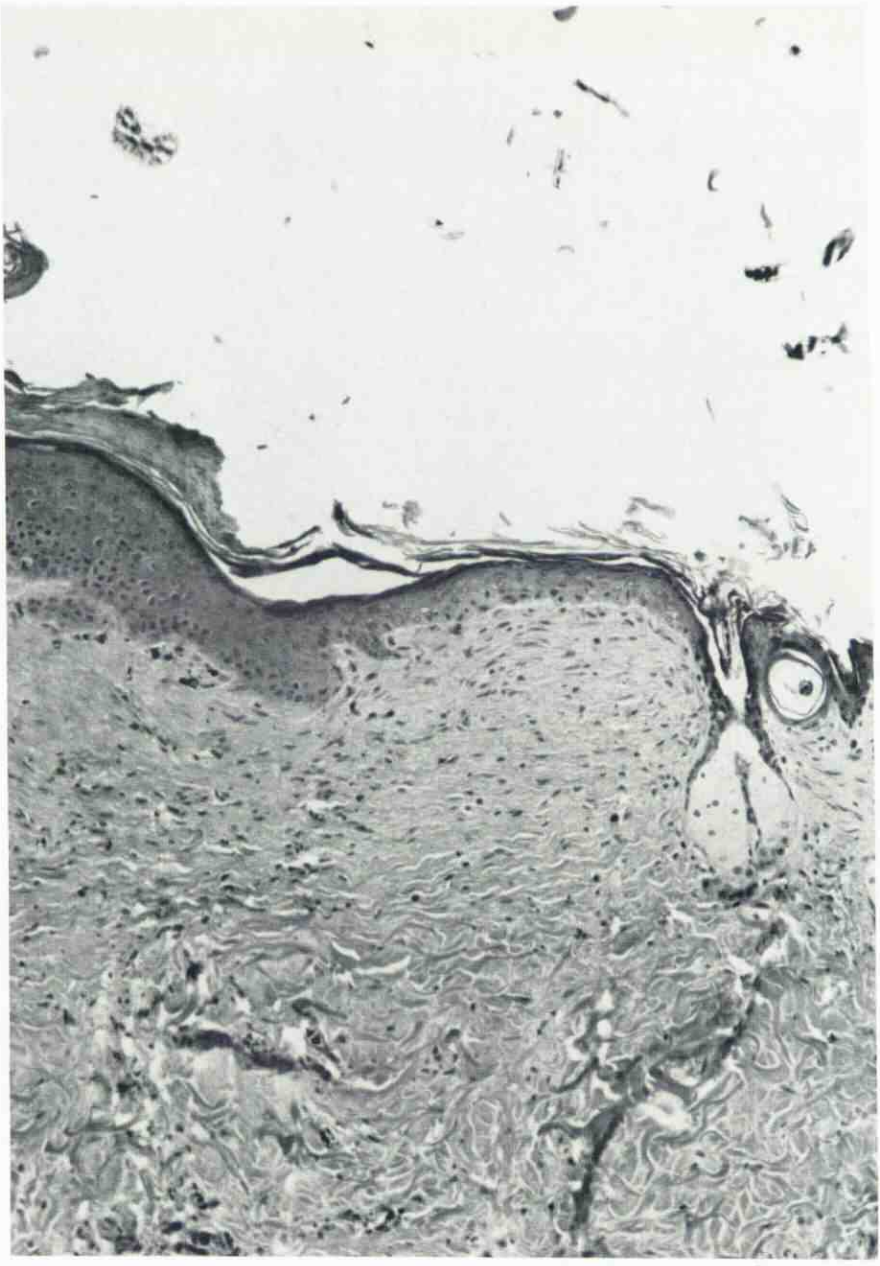

FIG. 3: Hematoxylin and eosin stained section at the edge of a xenograft 27 days after grafting. Human skin is on the left and mouse skin on the right. Original magnification × 200.

sacrificed and tissues histologically examined. The inoculum contained whole virions and clumped debris including bacterial forms. The graft site (Fig. 3) showed a transition from hair-bearing mouse epidermis only several cells thick to epidermis closely resembling normal human skin, except for a parakeratotic corneum and some effacement of rete ridges. Collagen fibers below the human epidermis were thicker and more densely organized than in mouse skin. No gross or histologic evidence of wart formation was observed. Mononuclear infiltrates were not evident. Microscopic examination of the viscera revealed only focal atelectasis and pneumonitis. There were reduced numbers of lymphocytes in the splenic parenchyma.

DISCUSSION

This report describes the prolonged survival of human skin xenografts for periods of time up to over eight months on ATS-treated mice. Others have reported the survival of human split-thickness xenografts for five weeks in antilymphocyte-treated mice [4]. The grafts in our experiments

remained in good condition for the life of the animal, affording a small but easily observed and manipulated area of human skin. The lack of mononuclear leukocytic infiltration suggests efficient suppression of cell-mediated immunity. However, the contracture of these grafts could be the result of chronic rejection, possibly on an antibody basis since antithymocyte serum does not completely abolish antibody formation [6].

The failure of the grafts to form verrucae may be secondary to the irregular infectivity and prolonged latency noted with the inoculation of wart virus in human volunteers [2]. We attempted to prepare an inoculum with a minimum of experimental manipulations which might inactivate the virus, but there is no way short of human inoculation to assay infectivity. An intriguing possibility accounting for the negative results is that the ATS might include neutralizing antibody to the human wart virus. Although mice do not encounter this virus spontaneously, it could be cross-reactive with other murine virions. This possibility cannot be directly tested in the absence of an infectivity assay for human wart virus.

In summary, some human skin grafts placed on ATS-treated mice survived at least eight months. Although we have been unable to produce verrucae on the graft site, the experimental technique is simple and we commend it to investigators interested in similar applications.

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