

INFLUENCE OF CULTURING ON THE SURVIVAL OF
MAJOR HISTOCOMPATIBILITY COMPLEX-COMPATIBLE
AND -INCOMPATIBLE THYROID GRAFTS IN RATS*

By STEPHEN T. BARTLETT, ANTHONY S. JENNINGS, CHU YU, ALI NAJI,
CLYDE F. BARKER, AND WILLYS K. SILVERS

*From the Departments of Surgery, Medicine, and Human Genetics, University of Pennsylvania School of
Medicine, Philadelphia, Pennsylvania 19104*

There is now ample evidence that the survival of endocrine allografts can be significantly prolonged if first maintained in culture. However, as far as we are aware, this has only been demonstrated when a major histocompatibility complex (MHC) barrier (1-7) or, even more surprisingly, a xenogeneic barrier (8-11) prevails. Indeed, the only experiments concerned with the fate of cultured MHC-compatible allografts, namely those with rat parathyroid glands, indicate that they survive no longer than fresh transplants (12). To explain these results, we have proposed (13) that allografts provoke a strong immune response only if they include donor macrophages or if MHC-compatible macrophages are available to react with cells bearing the foreign antigens. The clinical implications of this hypothesis are obvious, for, if substantiated, it suggests that transplants devoid of passenger cells might be more likely to survive in MHC-incompatible hosts. The macrophage seems likely to be the most important passenger cell because of its role in antigen presentation. Because our hypothesis is contrary to current thinking, it is important to determine whether what holds for parathyroid allografts holds for other transplants as well. Here we present evidence that cultured thyroid fragments also survive longer than fresh allografts only in MHC-incompatible rats.

Materials and Methods

Animals. Young adult (150-175 g) Lewis, Fischer, and ACI rats were employed. Lewis and Fischer rats are RT1¹ at the MHC and ACI animals are RT1^a. The Lewis and ACI animals were obtained from Harlan-Sprague Dawley, Walkersville, MD and the Fischer rats from Charles River Breeding Laboratories, Inc., Wilmington, MA. Thyroidectomized Lewis and ACI animals served as recipients for fresh and cultured Fischer thyroid tissue. Thyroidectomized Fischer rats received fresh and cultured isografts.

Thyroidectomy. All recipients were thyroidectomized under ether anesthesia through a mid-line cervical incision. Both lobes of thyroid gland were isolated and removed with care being taken not to damage the recurrent laryngeal nerves. Completeness of the thyroidectomy was confirmed by measurement of serum thyroxine (T₄) levels by radioimmunoassay (14). Only animals with serum thyroxine levels of ≤ 0.25 $\mu\text{g}/\text{dl}$ (normal rat serum T₄ concentration is >1.0 $\mu\text{g}/\text{dl}$), indicating an almost complete athyroid state, were used as recipients. All hosts were autopsied at the end of the experiment to confirm, both grossly and histologically, that thyroidectomy was complete.

* Supported by grants AM-26007, AM-19523, AM-30532, and CA-18640 from the National Institutes of Health.

Preparation of Grafts. The thyroid tissue to be grafted was procured as described above, except that the donors were pretreated with 300 mg/kg body weight of cyclophosphamide intraperitoneally 4 and 2 d before removal of the glands. This procedure promotes the removal of passenger leukocytes.

Excised thyroid lobes were cut into 1-mm³ fragments, and one donor equivalent of tissue was either grafted immediately under the kidney capsule of athyroid recipients or was transplanted to this site after 21 d in tissue culture. H-Y-incompatible grafts were avoided.

Culturing. The thyroid fragments were placed in 60-mm sterile tissue culture plates containing 3 ml of tissue culture medium (RPMI 1640 supplemented with glutamine [10 g/liter], penicillin/streptomycin/fungizone [10 ml/liter], and 15% isogenic rat serum). Culture plates were maintained in humidified gas boxes containing 95% O₂, 5% CO₂ at 37°C for 21 d. The culture medium was changed three times per week.

Evaluation of Grafts. Since we have found weight gain to correlate well with thyroid function, rats were weighed before thyroidectomy and at least once a week after receipt of grafts. T₄ levels were measured every other day in animals receiving fresh grafts and weekly in animals receiving cultured transplants. Grafts were considered rejected when T₄ levels returned to pretransplant levels. All grafts were biopsied and examined histologically after they had been in residence for 30 d. They were also appraised histologically, after sectioning and staining with hematoxylin and eosin, at the conclusion of the experiment.

Statistics. Median survival times (MST) of grafts were determined using a computer program employing probit transformation (15). Probability values were calculated with the Mann-Whitney U test.

Results

The results are summarized in Table I. As expected, all fresh isografts were accepted permanently. Recipients of such grafts displayed normal T₄ levels accompanied by progressive weight gain. When examined histologically after they had been in residence for >100 d, these grafts all displayed viable thyroid follicles and appeared normal in every respect. Cultured isografts produced similar results, indicating that 3 wk in culture had not adversely influenced their survival.

On the other hand, although both fresh MHC-incompatible and -compatible allografts functioned for as long as 4 wk, recipients of such grafts ceased to gain weight, and the grafts were ultimately rejected. Their MST in MHC-incompatible and -compatible hosts was 7.7 and 12.0 d, respectively.

Despite the shorter survival of fresh MHC incompatible than compatible grafts, cultured grafts functioned significantly longer in MHC incompatible hosts ($P = \leq 0.0002$). Thus, cultured Fischer thyroid fragments functioned for at least 21 d in 19 of 23 MHC-incompatible hosts (MST, 23.0 d), and in 4 hosts they functioned for >100 d. In fact, except for a small amount of mononuclear cuffing, these long-

TABLE I
Survival of Fresh and Cultured Fischer Thyroid Grafts in MHC-compatible (Lewis) and -incompatible (ACI) Hosts

| Graft | Recipient | Number | Survival | MST | Mean weight gain |
|----------|-----------|--------|--|------|------------------|
| | | | | | <i>g</i> |
| Fresh | Fischer | 12 | 12 × >100 | — | 141.0 |
| Cultured | Fischer | 8 | 8 × >100 | — | 152.0 |
| Fresh | ACI | 9 | 4 × 6, 8, 3 × 10, 28 | 7.7 | 16.3 |
| Cultured | ACI | 23 | 4 × 7, 6 × 21, 3 × 23, 3 × 28, 30, 2 × 40, 4 × >100. | 23.0 | 45.0 |
| Fresh | Lewis | 11 | 8, 10, 6 × 12, 14, 21, 28 | 12.0 | 6.6 |
| Cultured | Lewis | 22 | 9 × 7, 3 × 14, 4 × 21, 2 × 23, 2 × 28, 2 × 50 | 9.8 | 6.0 |

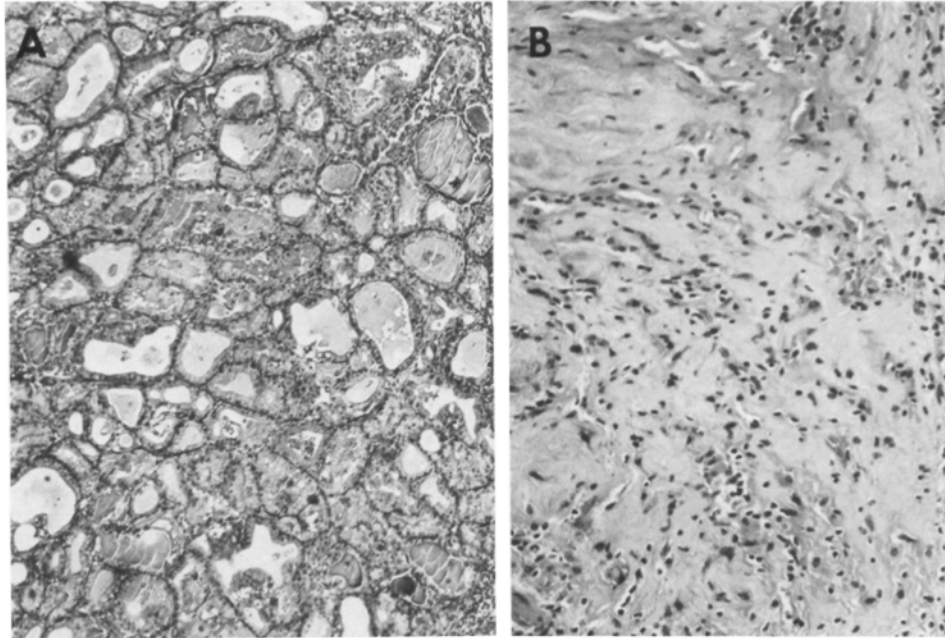


FIG. 1. (A) Cultured Fischer thyroid 150 d after renal subcapsular transplantation to MHC-incompatible ACI host ($\times 75$); and (B) 30 d after transplantation to MHC-compatible Lewis recipient ($\times 175$). Note normal follicular structure in (A) and absence of any viable graft in (B).

surviving grafts appeared as healthy as isografts when examined histologically at 150 d (Fig. 1 A). In contrast, cultured Fischer grafts functioned for 21 d in only 10 of 22 MHC compatible Lewis recipients, and in only 2 animals did they function for as long as 50 d, i.e., longer than any uncultured grafts. The MST of these cultured MHC compatible transplants was 9.8 d, an MST not significantly different ($P = 0.32$) from the MST of fresh Fischer grafts in Lewis hosts.

Histological appraisal of the grafts revealed striking differences between those residing in MHC-compatible and -incompatible hosts. For example, our first series of 30-d biopsies revealed that 10 of 11 cultured grafts had been completely obliterated in MHC-compatible hosts (Fig. 1 B). Similar grafts, although displaying varying degrees of mononuclear infiltration, some of which was intense, remained histologically intact in 8 of 11 MHC incompatible recipients.

Finally, as noted previously, body weight gain correlates well with thyroid graft function (see Table I). Whereas the mean weight gain in recipients of MHC-incompatible cultured grafts was significantly greater than those receiving fresh tissue ($P = 0.007$), rats receiving MHC-compatible cultured allografts failed to gain more weight than recipients of fresh compatible grafts ($P = 0.89$).

Discussion

Although we were able to procure the permanent survival of cultured thyroid allografts in only four recipients, all of these recipients were MHC incompatible with the graft. Moreover, as reported for parathyroid allografts (12), culturing only augmented the survival of MHC-incompatible thyroid grafts, having little, if any,

effect on the survival of MHC-compatible glands. Indeed, the difference in the effect that culturing had in promoting the survival of MHC-incompatible and -compatible transplants was more striking for thyroid than for parathyroid grafts. Whereas a significant percentage of fresh and cultured MHC-compatible parathyroid grafts survive indefinitely, MHC-compatible thyroid grafts, regardless of whether they had been cultured or not, were all rejected within 50 d. Our inability to obtain permanent survivals for more of the cultured MHC-incompatible grafts is most likely due to the persistence of some passenger lymphoid cells. In this regard, it has been reported that in mice, only 10^3 donor peritoneal exudate cells need be added to prejudice the survival of otherwise accepted putatively "passenger cell-free" MHC-incompatible thyroid grafts (16).

The results reported here provide further support for our contention that transplantation antigens are unlikely to be recognized in the absence of an MHC-compatible antigen-presenting cell, which may be of donor or host origin. More definitive evidence for such restriction must await the results of studies with the appropriate congenic strains. These are currently underway.

Summary

Culturing Fischer thyroid fragments promotes their survival in major histocompatibility complex (MHC) -incompatible ACI rats but not in MHC-compatible Lewis animals.

Received for publication 13 September 1982 and in revised form 19 October 1982.

References

1. Jacobs, B. B. 1974. Ovarian allograft survival. *Transplantation (Baltimore)*. **18**:454.
2. Lafferty, K. J., M. A. Cooley, J. Woolnough, and K. Z. Walker. 1975. Thyroid allograft immunogenicity is reduced after a period in organ culture. *Science (Wash. D. C.)*. **188**:259.
3. Lafferty, K. J., A. Bootes, G. Dart, and D. W. Talmage. 1976. Effect of organ culture on the survival of thyroid allografts in mice. *Transplantation (Baltimore)*. **22**:138.
4. Lafferty, K. J., A. Bootes, V. A. Killby, and W. Burch. 1976. Mechanism of thyroid allograft rejection. *Aust. J. Exp. Biol. Med. Sci.* **54**:573.
5. Keding, M., K. Haffen, J. Grenier, and R. Eloy. 1977. *In vitro* culture reduces immunogenicity of pancreatic islets. *Nature (Lond.)*. **270**:736.
6. Lacy, P. E., J. M. Davie, and E. H. Finke. 1979. Prolongation of islet allograft survival following *in vitro* culture (24°C) and a single injection of ALS. *Science (Wash. D. C.)*. **204**:312.
7. Bowen, K. M., and K. J. Lafferty. 1980. Reversal of diabetes by allogeneic islet transplantation without immunosuppression. *Aust. J. Exp. Biol. Med. Sci.* **58**:441.
8. Sollinger, H. W., P. M. Burkholder, W. R. Rasmus, and F. H. Bach. 1977. Prolonged survival of xenografts after organ culture. *Surgery*. **81**:74.
9. Sollinger, H. W., P. M. Burkholder, O. J. Kuperman, and F. H. Bach. 1977. Prolonged survival of xenografts after organ culture. *Transplant. Proc.* **9**:359.
10. Lacy, P. E., J. M. Davie, and E. H. Finke. 1980. Prolongation of islet xenograft survival without continuous immunosuppression. *Science (Wash. D. C.)*. **209**:283.
11. Lacy, P. E., J. M. Davie, and E. H. Finke. 1981. Prolongation of islet xenograft survival (rat to mouse). *Diabetes*. **30**:285.
12. Naji, A., W. K. Silvers, and C. F. Barker. 1981. Influence of organ culture on the survival of major histocompatibility complex-compatible and incompatible parathyroid allografts in rats. *Transplantation (Baltimore)*. **32**:296.

13. Silvers, W. K., H. L. Fleming, A. Naji, and C. F. Barker. 1982. Evidence for major histocompatibility complex restriction in transplantation immunity. *Proc. Natl. Acad. Sci. U. S. A.* **79**:171.
14. Chopra, I. J. 1972. Radioimmunoassay for measurement of thyroxin in unextracted serum. *J. Clin. Endocrinol. Metab.* **34**:938.
15. Finney, D. J. 1952. Probit Analysis. Cambridge University Press. 256.
16. Talmage, D. W., G. Dart, J. Radovich, and K. J. Lafferty. 1976. Activation of transplant immunity: effect of donor leukocytes on thyroid allograft rejection. *Science (Wash. D. C.)*. **191**:385.