CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY 33, 381–390 (1984)

Immunologic Effects of Human Thymic Stromal Grafts and Cell Lines[1]

ESTHER F. HAYS* AND TERRY R. BEARDSLEY†,[2]

*Department of Medicine, Laboratory of Biomedical and Environmental Sciences, University of California, Los Angeles, California 90024 and †Department of Biology, University of California, San Diego, California 92037

These experiments report a method of preparing stromal remnants from human thymus. The remnants were composed of epithelial cells, fibroblasts, and macrophages. They were grafted under the renal capsule of nude mice. Some of the grafts were reconstituted with lymphocytes to obtain the microscopic morphology of the thymus. The mice with reconstituted grafts survived in a conventional environment, had increased numbers of T cells in their spleen, and showed improvement of T-cell mediated immunologic function. This was measured by a positive allogeneic effect, a mixed lymphocyte reaction, and cell-mediated lysis. *In vitro* cell lines were established from thymic remnants of two separate individuals. These lines grew as attached monolayers. One of them was composed of fibroblasts. The other had an epithelial morphology. This epithelial cell line (HT-7) was shown to produce factors which promoted thymocyte differentiation *in vitro*. © 1984 Academic Press, Inc.

INTRODUCTION

The thymic stroma is known to contain epithelial cells and macrophages which function as a microenvironment for bone-marrow-derived T-lymphocyte progenitors. Previous studies using both syngeneic and allogeneic (allo) thymic stromal grafts in mice have shown that these grafts are populated with recipient lymphocytes and reconstituted to a new thymus at the site of the graft (1). Such thymic remnant grafts have been shown to result in improved T-cell function in nude mice (2, 3). Human thymic remnant grafts have been shown to have some beneficial effects in patients with immune deficiency syndromes (4). Preparation of thymic remnants has involved culture in *in vivo* diffusion chambers (5) or *in vitro* (6–8). Such treatment results in loss of thymic lymphocytes and persistence of stromal remnants which can, in syngeneic mice, reconstitute a morphologically normal thymus at the site of the graft. Remnants prepared from both old and young mice have been shown to have this capability (8).

This paper reports studies of human thymus remnants prepared by *in vitro* culture. We were able to obtain remnants which were reconstituted with host lymphocytes in the nude mouse. The grafted mice developed T-cell-dependent immunologic function. A continuous cell line was established from one of the

[1] Supported by the National Institutes of Health, Grant CA-12386, and the Department of Energy, Contract DE-AM03-76-SF00012.

[2] Present address: Beckman Instruments, Inc., Irvine, Calif. 92714.

0090-1229/84 $1.50
Copyright © 1984 by Academic Press, Inc.
All rights of reproduction in any form reserved.

human remnants. This line produced factors which resulted in *in vitro* T-cell maturation.

MATERIALS AND METHODS

Mice. BALB/c *nu/nu* female mice were from the specific pathogen-free (SPF) nude mouse breeding facility in Dr. Hays' laboratory. The BALB/c ($H2^d$), HW-19 ($H\text{-}2^d$), and B6-H-2^k mice which were used as stimulator spleen cells, sources of B cells, and controls were from the colony of Dr. Richard Dutton, University of California, San Diego.

Thymus remnant preparation. The thymus remnants were obtained from organs that had been removed at the time of cardiac surgery. The organs were placed in cold phosphate-buffered saline (4°C) and brought to the laboratory within 2–6 hr after their removal. The thymus was minced into 1-mm-sized pieces and 15 pieces were placed on a single Nucleopore-100 membrane floating in a 35-mm culture dish with 1 ml of Waymouth medium, 20% fetal bovine serum (FBS), and penicillin/streptomycin (P/S). The thymic pieces were placed at 24°C in a 5% CO_2 incubator for 1 week. After 1 week, the medium was removed and 1 ml of fresh medium added. The remnants were cultured for an additional 3 weeks at 37°C in 5% CO_2 in air with weekly medium changes.

At the end of the 4-week culture period, the remnants were removed from the membranes and grafted under the renal capsule of 8-week-old BALB/c *nu/nu* mice using ether anesthesia. Each mouse received approximately five remnant pieces. The surviving grafted nude mice were sacrificed 4 months after grafting. They were maintained in a conventional environment during that time period. Some remnants were saved and transferred to separate dishes to establish cell lines and others were fixed in Bouin's fluid and used for preparation of hematoxylin and eosin stained sections.

Establishment of cell lines. Remnant pieces were placed in 35-mm dishes with Waymouth medium, 20% FBS, and P/S at 37°C in a CO_2 incubator. They attached to the surface of the dish within 24 hr and cells could be seen growing from the remnant pieces after several days. These cells grew very slowly. The medium was changed once a week. When 3- to 4-mm outgrowths were seen from the remnant pieces, the pieces were removed with sterile forceps and the monolayer cells continued to spread. After about 4 weeks in culture the cells were trypsinized and seeded in fresh dishes at 1×10^6/ml. These cells then reached confluence at 1 week and were continued as a cell line with weekly or biweekly passage. They were maintained in Dulbecco's minimum essential medium (DMEM) with 10% FBS. Supernatant medium was harvested from cultures for the immunologic studies after the fourth and fifth passages in the following manner. When the cells reached confluence at 5 days, the growth medium was removed and replaced with serum-free DMEM for 24 hr. The 24-hr supernatant served as a source of medium for the CML assay.

Cell preparations. T lymphocytes were obtained from spleen and lymph node cells of normal BALB/c, BALB/c *nu/nu,* or grafted *nu/nu* mice. All were passed through nylon-wool columns (9).

Positive allogeneic effect (PAE) assay (10). The helper cell activity in the mixed

lymphocyte response (MLR) was measured by addition of varying numbers of mitomycin-treated T-cell preparations to a constant number of B cells (anti-Thy 1.2 plus complement-treated spleen cells) (6×10^5/well) in the presence of 0.1% (v/v) sheep red blood cells (SRBC). Cells were cultured for 4 days under standard microculture conditions after which the primary hemolytic plaque-forming cell (PFC) response was measured.

MLR. These studies were done in flat-bottom microtiter wells. Appropriate numbers of nude mouse T cells were pipeted into microwells in 0.1-ml volumes. Corresponding numbers of irradiated (1500 rad) stimulator cells were also added to wells in 0.1-ml volumes. Maximal stimulation occurred when 0.6×10^5 cells were cultured with an equal number of stimulator cells. Stimulator cells were HW-19 ($H\text{-}2^d$) or B6-$H\text{-}2^k$. Cultures were incubated at 37°C in humidified 5% CO_2–95% air for 42, 66, and 96 hr, including an 18-hr pulse label with 2 μCi (0.05 ml) of [^{3}H]thymidine. Maximal responses occurred at 66 hr. Cultures were harvested using a multiple automated sample harvester (MASH II) (Microbiological Associates, Los Angeles, Calif.) and prepared for scintillation counting. Data from five separate experiments were averaged, and standard errors were computed.

Cell-mediated lympholysis (CML). T cells (5×10^6) were cultured in RPMI 1640 plus 10% FBS with 4×10^6 mitomycin-treated spleen cells in 60-mm dishes. Cultures were incubated for 5 days at 37°C in a humidified 5% CO_2 atmosphere with continuous rocking. Cells were tested after 5 days for cytotoxicity against the appropriate ^{51}Cr-labeled target cells. Mouse spleen cells which had been stimulated for 48 hr with concanavalin A (Con A) (2 μg/ml) served as targets for the CML of the *nu/nu* spleens as well as for the thymocytes preincubated in supernatant from the thymic stromal cell lines. Varying numbers of effector cells were incubated for 4 hr with 2×10^4 target cells. The percentage of specific isotope release was calculated according to the standard formulation (11). CML of nude mouse spleens used 8×10^6 T cells from nylon-wool effluents. CML induced by conditioned media of cell lines was carried out using 5×10^6 thymocytes separated from whole thymus preparations with peanut agglutinin (PNA) according to the method of Kruisbeck and Astaldi (12). An analysis of the PNA^+ population by staining with fluorescein isothiocyanate (FITC)-PNA and using a fluorescence-activated cell sorter (FACS-IV) indicated <4% contamination by PNA^- or low fluorescent cells. The PNA^+ thymocytes were cultured in 2 ml of RPMI 1640 with 5%, FBS, glutamine, penicillin/streptomycin, and 2-mercaptoethanol (50 μ*M*) in 24-well plates. Mitomycin-treated spleen cells (5×10^6) were added and the cell mixture was incubated for 5 days at 37°C. The medium used in these assays was RPMI 1640 or conditioned RPMI 1640 from the thymic stromal cell lines. Fresh serum and the other ingredients were added to these sources of medium for the assay. After 5 days, the cells were tested for killing by a 4-hr incubation with ^{51}Cr-labeled Con A blast targets as described above.

Interleukin 2 (IL-2)assay. Supernatants from the PAE and MLR cultures were tested for IL-2 action by using the CTLL-2 T-cell line (13). CTLL-2 cells 5×10^3 were incubated at 24 hr with various dilutions of supernatant. Proliferation was measured by labeling the cells with ^{125}I-dUrd for the last 4 hr of culture.

RESULTS

Survival of Nude Mouse Recipients

A total of 14 nude mice were grafted under the renal capsule with thymic remnants prepared from six separate patients (Table 1). One patient was a 19-year-old female; the remaining five thymuses came from two males and three females, ages 8–17 months. All of the mice were moved to a conventional environment for the grafting procedure. Subsequently, they were housed in a conventional environment, but in filter-top cages and given autoclaved food and water for 2 months. The survivors were then shipped to San Diego and for the next 2 months reared in a conventional environment with standard food and water. Of the mice receiving grafts, 8 animals were long-term survivors. They maintained their weight and vigor for 4 months after grafting when they were killed for immunologic studies. The 6 animals that died were from the HT-2, HT-3, and HT-5 experiments. They died after showing wasting and inanition within 2 weeks after being moved to the conventional environment. Grafts were not visible at autopsy. The microscopic appearance of the remnants from HT-2 and HT-3 preparations revealed necrosis or remnants with very few viable cells; i.e., the cells did not survive the *in vitro* conditions. In contrast, the microscopic morphology of the cultured remnants from the HT-1, -4, -5, and -7 showed epithelial cells, fibroblasts, and macrophages. Some epithelial cells were arranged in clusters or had an acinar appearance. Grafts in the 8 long-term survivors could be seen as slightly raised, 1- to 3-mm, white areas near the upper pole of the kidney. The microscopic morphology of these grafts showed some variation. The HT-1 and HT-7 grafts showed reconstitution, i.e., lymphocyte repopulation with corticomedullary distinction. In contrast, the HT-4 graft and the HT-5 remnant graft from the one survivor were composed principally of epithelial cells and macrophages. Only small areas of lymphoid cells were seen in each of these grafts. It should be noted that the thymus specimen from HT-2, HT-3, HT-4, and HT-5 remained in cold PBS for 6 hr prior to mincing and placement in growth medium at 24°C. Thymus specimens from HT-1 and HT-7 were minced and placed in growth medium within 2 hr after removal from the patient.

TABLE 1
SUMMARY OF HUMAN THYMUS REMNANTS USED IN THE STUDIES

	Donor		No. mice		No. mice	
	Sex	Age	grafted	Survivors[a]	studied	Histology of graft
HT-1	F	19 years	3	3	3	Lymphoid reconstitution
HT-2	M	12 months	3	0	0	No graft seen
HT-3	F	10 months	2	0	0	No graft seen
HT-4	F	8 months	1	1	1	Few lymphocytes
HT-5	F	10 months	2	1	1	Few lymphocytes
HT-7	M	17 months	3	3	3	Lymphoid reconstitution

[a] Survivors were animals living 4 months in a conventional environment.

Numerical and Functional in Vitro Tests

Findings regarding T-cell number and function as measured by *in vitro* tests are reported from the eight surviving mice. These consisted of three animals receiving HT-1 grafts, one mouse receiving an HT-4 graft, one mouse receiving an HT-5 graft, and three mice receiving HT-7 grafts (Table 1). When 100×10^6 normal mouse spleen cells were passed through nylon-wool columns, 35% of the cells were recovered in the effluent and were shown to be Thy-1-positive T cells. When cells from a nude mouse spleen which has a comparable weight to conventional mouse spleen were passed through a nylon-wool column, only 3% of the cells were recovered. These cells were Thy 1 negative. The cell numbers recovered from passage through nylon-wool columns in the nude mice with human thymus remnant grafts compared to those from normal BALB/c or nude mice are presented in Table 2. In each instance, 100×10^6 spleen cells were added to the column. The recovered cells were used in the functional assays to be described subsequently. It can be seen from the table that the nude mice with human thymus remnant grafts produced more nylon-wool nonadherent cells than the nude mouse controls and some were in the same range as a normal BALB/c mouse. Furthermore, the recovered cells from the nude mice with grafts and the BALB/c mice were Thy 1.2 positive indicating that they were T cells and, in the case of the nude mice, derived from the mouse host. The recovered *nu/nu* spleen cells were Thy 1.2 negative.

Table 3 and Fig. 1 present the results of *in vitro* immunologic studies on the survivors. They show that some animals with HT-1 and HT-7 grafts developed T-cell-dependent immunologic function as measured by PAE, MLR, and CML. The CML reactions and the PAE reactions of two HT-1 and two HT-7 grafted mice showed responses to $H\text{-}2^k$ cells and not to $H\text{-}2^d$ cells (Fig. 1). However, the PAE and MLR reactions of one HT-1 and two HT-7 recipients showed responses to $H\text{-}2^d$ cells as well as to $H\text{-}2^k$ cells (Table 3). This apparent self reactivity in

TABLE 2

NUMBER OF CELLS RECOVERED FROM THE SPLEEN AFTER PASSAGE OF 100×10^6 CELLS THROUGH A NYLON-WOOL COLUMN

BALB/c	56×10^6
	40×10^6
	30×10^6
nu/nu	4×10^6
	6×10^6
nu/nu grafted with	
HT-1	15×10^6
	43×10^6
HT-4	7×10^6
HT-5	32×10^6
HT-7	35×10^6
	28×10^6

TABLE 3
RESULTS WITH POSITIVE ALLOGENEIC EFFECT (PAE) AND MIXED LYMPHOCYTE REACTION (MLR) OF T CELLS FROM NUDE MICE WITH XENOGRAFTS OF HUMAN THYMUS EPITHELIUM

Source of T cells	PAE (B cells)		MLR (Stimulator cells)	
	HW-19(H-2^d)	B6-H-2^k	HW-19(H-2^d)	B-6-H-2^k
	PFC/culture		cpm	
BALB/c	37 ± 8.2[a]	2283 ± 295.7	454 ± 308	5326 ± 432
nu/nu HT-1				
(1)	19 ± 5.7	264 ± 3.7		
(2)	173 ± 12	2	—	—
(3)	568 ± 48.1	1685 ± 418		
nu/nu HT-4	10 ± 6	25 ± 10	1377 ± 69.5	1302 ± 693
nu/nu HT-5	0	30 ± 9.6	253 ± 8.5	343 ± 21
nu/nu HT-7				
(1)	718 ± 4.7	208 ± 7.5	3040 ± 200	2348 ± 142
(2)	54 ± 4.3	1365 ± 87	2113 ± 416	3256 ± 126
(3)	306 ± 20.8	1559 ± 125.3	—	—
nu/nu	0.7 ± 0.3	246 ± 18.9	—	—
	0	0	—	—

Note. MLR were not done on nude mouse cells because there were not enough cells available from a single spleen to perform the assay. The results with *nu/nu* HT-1 and *nu/nu* HT-7 represent data from three individual mice receiving the thymus remnant grafts.

[a] Standard error.

nude mice with thymic grafts has been seen in other mouse systems (14, 15). The responses in HT-1 and HT-7 correlated with the T-cell numbers recovered from the spleen and the reconstituted appearance of the graft. The HT-4 and HT-5 results showed a general lack of reactivity (except slight reactivity in HT-4 MLR to both H2-^{d} and H-2^k). It is of interest to note that HT-5 although showing a graft with a minimal lymphoid population and abnormal T-cell function had a "normal" number of T cells recovered after passage of the spleen through nylon wool (Table 2).

The production of IL-2 by the MLR supernatants was tested. In each case, the production of IL-2 correlated with the positive MLR activity (data not shown).

Cell Lines Established from HT Remnants

Continuous attached cell lines were established from the thymic remnants HT-4 and HT-7 as outlined under Materials and Methods. The cells grew as contact-inhibited monolayers. The cultures were examined in the living state by inverted phase contrast microscopy. The HT-4 cells were typical elongated fibroblasts while the appearance of HT-7 cells was quite different. They strikingly resembled the epithelial cells of the mouse TEPI 1 line previously described (16). Both cell lines had a normal human karyotype.

One prominent intrathymic differentiative event is the transition from a non-MLR reactive cell to a highly MLR-competent T cell. Presumably, this reflects

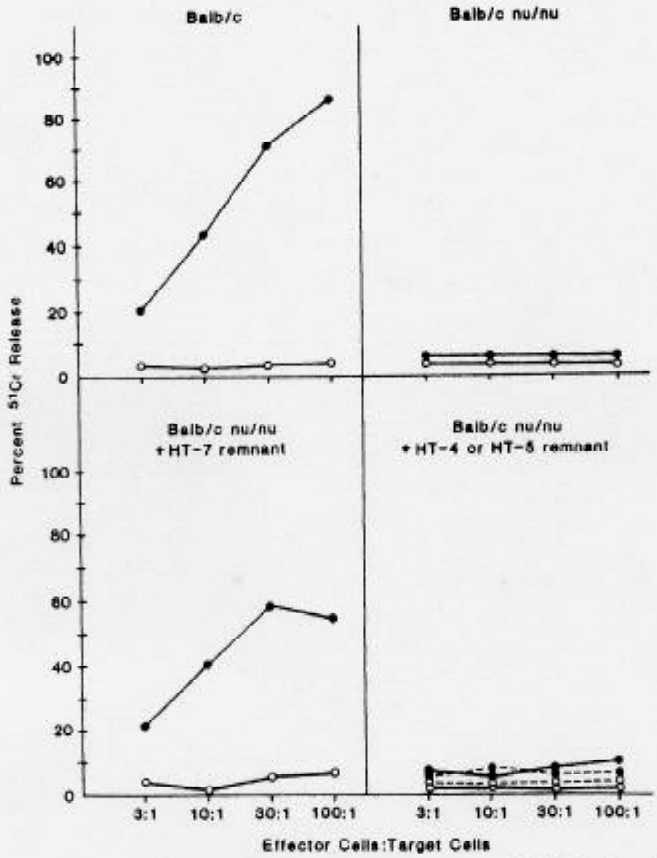

FIG. 1. CML reactions in nude mice with functional human xenograft HT-7 and nonfunctional human xenografts HT-4 and HT-5 (—), compared with CML reactions of BALB/c and BALB/c *nu/nu* mice. H-2^k targets (●); H-2^d targets (○).

the maturation of helper-T-cell function, which then can provide the already present CML precursors with the requisite signal to develop into killer T cells. In order to determine if our human stromal cell lines produced factors promoting this type of thymocyte differentiation, their conditioned media were evaluated. The results of this study are illustrated in Fig. 2 and show an increase in the amount of CML for H-2^d Con A blasts in a population of C57Bl/6 (H-2^b) PNA^+ thymocytes after 5 days in culture in the presence of conditioned media from HT-7. This was comparable to that of the two mouse thymic stromal lines, TEPI I or TEPI II (16). However, medium from the HT-4 line was similar to that of the control (test thymocytes incubated in normal growth medium) in its lack of augmentation of CML. This observation correlates with our findings of the development of functional T cells in the nude mice grafted with HT-7 but not with HT-4 as well as with the microscopic appearance of the monolayers; i.e., the cells producing the "active" conditioned medium had an epithelial morphology (HT-

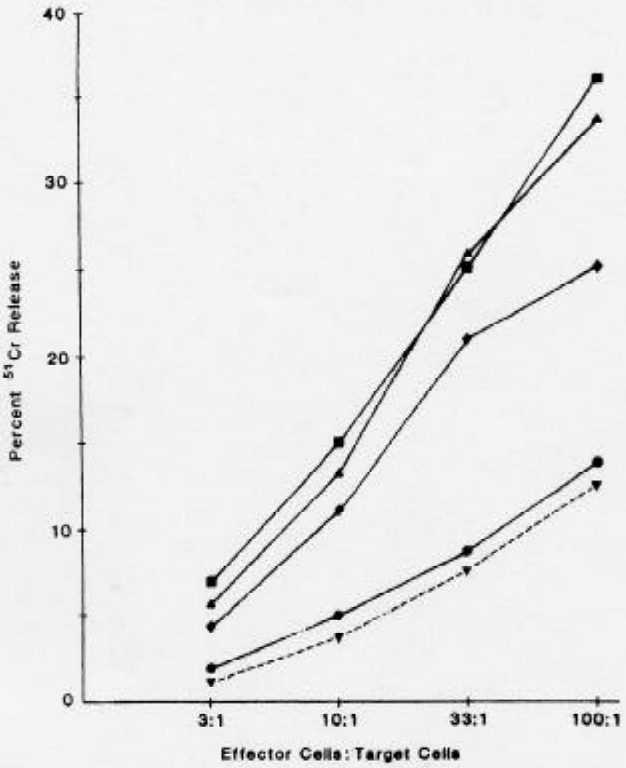

FIG. 2. This figure shows the results for augmentation of the CML response to an allogeneic major histocompatibility complex antigen. The responses of PNA^+ thymocytes in the presence of TEPI I (■); TEPI II (▲); HT-7 (◆); and HT-4 (●); supernatants (10% v/v). The response of cells without additions to the culture is indicated by (▼). The targets were spleen cells from BALB/c mice which had been stimulated for 48 hr with Con A (2 μg/ml).

7) whereas the supernatants from the fibroblast cultures of HT-4 demonstrated no functional activity in this assay.

DISCUSSION

These studies show that remnants prepared from a human thymus can effect immunologic reconstitution of nude mice. These mice had prolonged survival in good health in a conventional environment, and animals grafted with HT-1 and HT-7 showed development of immune function in their spleen cells as measured by *in vitro* tests. We used the PAE, MLR, and CML tests to determine functional reconstitution because these are tests of *in vitro* functional capability of T cells. This is in contrast to mitogen assays which measure a nonspecific response. The PAE was chosen because it is a more quantitative method for measuring the proliferative phase of the MLR, i.e., Ia-region recognition. The PAE reaction elaborates a factor to help B cells respond to SRBC. This factor production is influenced by recognition of Ia antigen on the target cell. The MLR and CML are standard tests of T-cell function. The animals grafted with HT-1 and HT-7

showed no thymic tissue in the mediastinum but exhibited morphologically reconstituted grafts. They survived in a conventional environment. Two mice grafted with HT-4 and HT-5 remnants had a prolonged survival with poorly reconstituted grafts and negative immunologic test results. The observation of prolonged survival in these animals suggests a partial response which was not detected by the assays used in this study. The remnants HT-2 and HT-3 had few viable cells after culture and, as expected, these grafts did not take and their hosts could not survive in a conventional environment.

A cell line with epithelial morphology was established from HT-7. This line was shown to produce factors which could augment allo-CML reactivity of PNA^+ thymocytes. These findings were similar to those observed in the mouse thymic stromal line TEPI (16). A line established from HT-4 remnants was composed principally of fibroblasts and did not produce thymic maturation factors.

Previously studies have reported that 1-week cultured syngeneic and allogeneic mouse thymic grafts under the kidney capsule of nude mice resulted in animals which would survive in a conventional environment (10 months). They acquired T cells of host origin which had demonstrable killer and helper capability (2). This same group of investigators has shown that transplantation of xenogeneic (rat) thymic remnants can restore cellular and humoral immunity in nude mice (17). It is of interest that the culture methodology described by the above studies yielded grafts with decreased function if the organs were cultured for more than 8 or 10 days at 37°C. However, an initial week of culture at 24°C was not used in these studies.

To address the question of the findings in the two animals with HT-4 and HT-5 grafts, we refer to the work of Crouse *et al.* (18), who described 14-day cultures of mouse fetal thymus grown for 7 days at 24°C and 7 days at 37°C. They determined that these cultures contained ectodermally-derived epithelium. The hosts were adult thymectomized, irradiated, fetal liver-reconstituted animals. Although both syngeneic and allogenic grafts showed lymphoid repopulation, no functional reconstitution of the T-cell deficient animals was obtained. These authors suggest that an endodermally-derived epithelial population is necessary for complete functional reconstitution in this system. These two germ layer sources of thymic epithelial cells have been described previously (19). These observations raise the possibility in our studies that the HT-4 and HT-5 remnants had only the ectodermally-derived components and hence could not achieve the degree of morphologic restoration and functional capability of the nude recipients seen with the HT-1 and HT-7 grafts. In addition, it should be pointed out that a circumstance which may account for the differences found when HT-1 and HT-7 grafts were compared with HT-4 and HT-5 grafts is that the former thymus tissues were minced and placed in growth medium 4 hr sooner than the latter. This may have allowed survival of the stromal cell population responsible for the reconstitution of HT-1 and HT-7 remnants.

The studies reported here are the first to describe the effect of grafting human thymus remnants into nude mice and show a variation in the capability of such remnants to function. They also describe a continuous cell line which was established from the same stromal cells which gave rise to functional grafts. This line (HT-7) produces factors which promote *in vitro* thymocyte differentiation.

ACKNOWLEDGMENTS

Our thanks to Dr. Helga Muller for performing the chromosome analysis of the human cell lines and to Donna Ricchiuti for typing the manuscript.

REFERENCES

1. Hays, E. F., *J. Exp. Med.* **129**, 1235, 1969.
2. Hong, R., Schulte-Wisserman, H., Jarrett-Toth, E., Horowitz, S., and Manning, D., *J. Exp. Med.* **149**, 398, 1979.
3. Manning, J. K., and Hong, R., *Thymus* **5**, 407, 1983.
4. Hong, R., Schulte-Wissermann, H., Horowitz, S., *et al.*, *Transplant. Proc.* **10**, 201, 1978.
5. Hays, E. F., *Cancer Res.* **28**, 21, 1968.
6. Shulte-Wissermann, H., Manning, D., and Hong, R., *Scand. J. Immunol.* **8**, 387, 1978.
7. Jordan, R. K., and Crouse, D. K., *In* "Development and Differentiation of Vertebrate Lymphocytes" (J. D. Horton, Ed.), Vol. 8, pp. 47–61, Elsevier/North-Holland, New York, 1980.
8. Hays, E. F., Swanson, S. K., Hale, L., and Margaretten, N., *Leuk. Res.*, in press.
9. Julius, M. H., Simpson, E., and Herzenberg, L. S., *Eur. J. Immunol.* **3**, 645, 1973.
10. Panfili, P. R., and Dutton, R. W., *J. Immunol.* **120**, 1847, 1978.
11. Matzinger, P., and Mirkwood, G., *J. Exp. Med.* **148**, 84, 1978.
12. Kruisbeek, A. M., and Astaldi, G. C. B., *J. Immunol.* **123**, 984, 1979.
13. Gillis, S., Ferm, M. M., Ou, W., and Smith, K. A., *J. Immunol.* **120**, 2027, 1978.
14. Beardsley, T. R., Swain, S. C., and Dutton, R. W., *In* "Mechanisms of Lymphocyte Activation (K. Resch and H. Kirchner, Eds.), p. 384. Elsiever/North-Holland, Amsterdam, 1981.
15. Clayberger, C., Dekruyff, R. H., and Cantor, H., *J. Immunol.* **132**, 2237, 1984.
16. Beardsley, T. R., Pierschbacher, M., Wetzel, G., and Hays, E. F., *Proc. Natl. Acad. Sci. USA* **80**, 6005, 1983.
17. Manning, J. K., and Hong, R., submitted for publication.
18. Crouse, D. A., Jordan, R. K., and Robinson, J. H., *Fed. Proc.* **41**, 605, 1982.
19. Jenkinson, E. J., Van Ewijk, W., and Owen, J. J. T., *J. Exp. Med.* **153**, 280, 1981.

Received April 17, 1984; accepted with revisions July 16, 1984.