Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 5663–5667, August 1986
Immunology

GH_3 pituitary adenoma cells can reverse thymic aging in rats

(thymus gland/immunophysiology/neuroimmunomodulation/T cells/immune response)

KEITH W. KELLEY*, SUSAN BRIEF*, HOLLIE J. WESTLY*, JAN NOVAKOFSKI†, PETER J. BECHTEL†, JOSEPH SIMON‡, AND EDWIN B. WALKER§

Laboratories of *Immunophysiology and †Muscle Biology, Department of Animal Sciences, College of Agriculture, and ‡Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801; and §School of Medicine, Indiana University, Indianapolis, IN 46202

Communicated by Glenn W. Salisbury, April 11, 1986

ABSTRACT **Thymic size and T-cell function decrease with age, and it has not yet been possible to totally reverse this thymic atrophy and completely restore T-cell-dependent immune functions. In this study, GH_3 pituitary adenoma cells, which secrete growth hormone and prolactin, were implanted subcutaneously into 16- and 22-month-old female Wistar-Furth rats and the rats were sacrificed approximately 2 months later. Only thymic remnants were detected in aged, non-implanted rats, but thymus glands were found in both the 18- and the 24-month-old rats that had been implanted with GH_3 cells. Thymus glands from the GH_3-implanted 18-month-old rats contained distinct cortical thymocytes and medullary epithelial cells. Depending on the concentration of phytohemagglutinin or concanavalin A, T-cell proliferative responses of splenocytes from these implanted rats were 2- to 5-fold greater than those of 18-month-old controls. At the optimal concentration of mitogen, proliferative responses to either lectin could be restored to those levels observed in splenocytes from 3-month-old Wistar-Furth females. Thymus glands from 24-month-old GH_3-implanted rats contained more cortical thymocytes and fewer fat vacuoles than controls, but they were not totally reconstituted. No significant lectin-induced T-cell proliferative responses or IL-2 secretion were detected in 24-month-old control rats, but splenocytes from GH_3-implanted rats showed augmented T-cell proliferative responses and increased synthesis of IL-2. Fluorescence-activated cell-sorter analysis of thymocytes revealed that 24-month-old rats implanted with GH_3 cells had a higher proportion of lymphocytes with the Thy-1.1 and helper-T-cell phenotypes. These data show that it is possible to regenerate normal thymic tissue *in situ* and reverse the natural loss in cell-mediated immunity that occurs with aging.**

Thymic size and T-cell function decrease with age, as measured by diminished T-cell proliferative responses to mitogens, reduced serum levels of thymic hormones, and lowered secretion of interleukin 2 (IL-2) (1–4). Thymic atrophy precedes the decline in T-cell proliferation, and thymic implants or thymic hormones can partially restore the diminished T-cell proliferation that occurs during aging (5–7). For these and other reasons, it has been argued that the thymus gland is the clock for immunologic aging (2, 8, 9). However, it is not known why the thymus gland involutes with age. Human fibroblasts that are cultured *in vitro* have a finite life span (10), so perhaps there is a clonal exhaustion of progenitor T cells in the bone marrow that migrate into the thymus. Aging may also lead to alterations of DNA within thymocytes. Another possibility is that extrinsic factors, such as hormones, are not secreted in adequate quantities to maintain either thymus size and/or T-cell function in aged individuals (8, 11, 12).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

If it were possible to augment thymic growth in aged animals, lectin-induced T-cell proliferation might also be restored. Until now, it has not been possible to totally reverse thymic atrophy or completely restore T-cell-dependent immune events in aged individuals. This problem reflects the lack of understanding about physiologic mechanisms that are responsible for involution of the thymus gland. However, it is well known that thymic size and growth hormone secretion are maximal at puberty (13–15) and that both of these decline with advancing age (3). Furthermore, treatment of young animals with pituitary extracts or growth hormone augments thymic size (16–19). Therefore, we hypothesized that pituitary hormones might restore normal thymic size, structure, and function in aged rats. Reconstitution with an autologous thymus gland might reverse the age-associated loss in T-cell-mediated immunity.

In this report, we demonstrate that GH_3 pituitary adenoma cells, which secrete both growth hormone and prolactin, can indeed reconstitute thymic structure and improve T-cell function when implanted into aged rats. These data indicate that lymphoid cells in aged animals are not inherently defective but can be restored with GH_3 cells.

MATERIALS AND METHODS

Rats. Wistar-Furth females that were 3 months old with an average body weight of 100 g or 16- and 22-month-old females with an average body weight of 240 g were housed under standard conditions with a 12/12-hr light/dark cycle. Wistar-Furth females have an average life span of 21 months (20).

GH_3 Cells. Three million GH_3 cells (American Type Culture Collection) grown in Ham's nutrient mixture F-10 with 5% fetal bovine serum and 10% horse serum were washed and injected subcutaneously in a volume of 0.5 ml. This pituitary epithelial cell line was derived from a Wistar-Furth rat and contains cells that secrete both growth hormone and prolactin (21, 22).

Histology. Thymus glands were sectioned (4 μm) and stained with hematoxylin and eosin.

Blood Cell Determination. Concentrations of erythrocytes and leukocytes in whole blood were determined with a Coulter ZM cell counter. Whole blood smears were also prepared and stained with Camco Quik Stain (American Scientific Products, Stone Mountain, GA) to determine the proportions of various leukocytes.

Mitogenesis. Rats were killed and lymphoid tissue was removed at approximately 60 days after the GH_3 cells were implanted, which is the time when maximal growth hormone can be detected in serum of GH_3-implanted animals (23). Spleen single-cell suspensions were prepared and erythrocytes were lysed with 0.83% NH_4Cl in 0.1% $KHCO_3$/0.01 mM EDTA. Splenocytes were suspended at 2.5×10^6 per ml in RPMI 1640 medium (pH 7.4) supplemented with 5% heat-inactivated fetal bovine serum, 100 units of penicillin per

Abbreviations: IL-2, interleukin 2; PHA, phytohemagglutinin.

ml, 100 μg of streptomycin per ml, and 24 mM $NaHCO_3$. One hundred microliters of cells was incubated with 100 μl of various concentrations of the mitogens Con A (Sigma) or phytohemagglutinin (PHA-M, GIBCO) in triplicate cultures at 39°C, 7% CO_2 for 72 hr. One microcurie of [*methyl*-^{3}H]thymidine (6.7 Ci/mmol; 1 Ci = 37 GBq) then was added to each well. Eighteen hours later, cells were harvested onto glass-fiber disks and dried. Three milliliters of scintillation fluid was added and radioactivity was measured in a Beckman LS 5801 liquid scintillation counter.

IL-2 Production and Measurement. Equal volumes of splenocytes (2.5×10^6 per ml) and Con A (5 μg/ml) or medium alone (control) were incubated for 48 hr. Cell-free supernatants were prepared by centrifugation and assayed for IL-2 activity on an IL-2-dependent murine cell line (24). Probit analysis was used to convert proliferative activity into units of IL-2 activity relative to a standard supernatant derived from Con A-activated rat splenocytes.

Flow Microfluorimetry. Single-cell suspensions were prepared from the thymus gland, spleen, and bone marrow. These preparations were washed three times in RPMI 1640 medium, and one million cells were incubated with 25 μl of rat IgG (1 mg/ml) for 15 min to block binding of Fc receptors. Fifty microliters of one of the following murine monoclonal antibodies (Pel-Freez) was then added: W3/13 (T cells), W3/25 (helper T cells), OX8 (T cells, non-helper subset), OX7 (Thy-1.1) and OX6 (Ia common determinant). After a 30-min incubation at 4°C, cells were washed three times with 0.15 M NaCl/10 mM phosphate, pH 7.4, containing 0.5% bovine serum albumin and 0.02% NaN_3. All cell suspensions were then incubated for 30 min at 4°C with 50 μl of a 1:20 dilution of fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (heavy- and light-chain specific) from HyClone (Logan, UT) that had been absorbed against rat IgG. Cells were washed three additional times. Cells from each animal were incubated separately with the secondary reagent control to determine background staining. At least 10^4 cells were counted with the FACS 440 laser flow-cytometry system (Becton Dickinson), using logarithmic amplification after adjustment of windows for background immunofluorescence.

Statistics. Data were subjected to analysis-of-variance procedures, and the pooled variance was used to calculate the standard error.

RESULTS

Histology. Thymus glands from 3-month-old rats ($n = 6$) averaged 242 ± 20 mg (mean ± standard error). Normal thymic histology was observed in these young rats (Fig. 1*A*). There was a clear demarcation of cortical and medullary regions, and at higher magnification (not shown), cortical thymocytes and medullary epithelial cells were identified. In both the control 18- and the control 24-month-old rats, only thymic remnants were detected (Fig. 1*B*). These thymus glands lacked a clear demarcation between the cortex and medulla, the interlobular septa were broad, and most of the parenchyma was filled with fatty tissue. Plasma cells were occasionally observed. There was much more thymic tissue (208 ± 21 mg, $n = 4$) in the 18-month-old rats that had been implanted 2 months earlier with GH_3 cells. These thymus glands contained distinct cortical and medullary areas (Fig. 1*C*) that were similar to thymic tissue of 3-month-old rats. Narrow interlobular trabeculae defined each of the thymic lobes. Large numbers of cortical thymocytes were present in the parenchyma. At higher magnification (not shown), Hassall corpuscles were identified in the thymic medulla. In the 24-month-old rats implanted 2 months earlier with GH_3 cells, larger thymus glands (200 ± 36 mg, $n = 6$) were observed than in the age-matched controls. Many more thymocytes colonized the cortex, and fewer fat vacuoles were found (Fig. 1*D*). However, developing cortical and medullary areas and the existence of some fat vacuoles within the interlobular trabeculae suggested that thymic restoration was not complete.

Mitogenesis and IL-2 Synthesis. Since rats with GH_3 tumors had larger thymus glands that appeared to have a normal histological appearance, it was important to determine whether T-cell mitogenesis was enhanced as well. As an index of the suppression in T-cell proliferation associated with aging, spleen cells were prepared from 3-month-old Wistar-Furth rats and incubated with several concentrations of two different T-cell mitogens, Con A and PHA (Table 1). In 18-month-old rats, there was an 80–90% reduction in proliferative responses of splenocytes to optimal concentrations of either Con A or PHA. No significant T-cell proliferative activity was detected in splenocytes from 24-month-old rats. Similarly, IL-2 concentration was very low in supernatants from cultured splenocytes of 24-month-old rats. This latter finding agrees with earlier studies of aged humans and mice, in which lower levels of IL-2 were produced as compared to younger animals, but conflicts with other reports (28, 29) in which IL-2 production was apparently normal in aged Lewis rats. When splenocytes from rats in each of the three age groups were cultured in the absence of mitogen (background), the average [^{3}H]thymidine incorporation was 700 cpm, and these values did not differ ($P > 0.10$) after GH_3 cells were implanted.

Eighteen-month-old rats that had been implanted with GH_3 cells displayed a 4-fold increase ($P < 0.01$) in the concentration of blood leukocytes (Table 1), with no change in the percentage of lymphocytes (68% for 18-month-old controls and 69% for similarly aged, GH_3-implanted rats). When equal numbers of splenocytes from 18-month-old control and GH_3-implanted rats were cultured with Con A and PHA, cells from the GH_3-implanted rats displayed significantly greater proliferative responses at almost every concentration of mitogen (Table 1). Maximal response to PHA from 18-month-old rats implanted 2 months earlier with GH_3 cells (13,900 cpm) was similar to PHA-induced proliferative responses of normal 3-month-old rats (12,700 cpm). Although there was a shift in the dose of Con A needed to cause maximal proliferation of splenocytes from 3-month-old vs. 18-month-old rats, the maximal response was not different between the two groups (32,100 cpm vs. 27,600 cpm).

When GH_3 cells were implanted into 24-month-old rats, there was also a 3-fold increase ($P < 0.10$) in the concentration of leukocytes in blood (Table 1) and no change in the percentage of lymphocytes (61% in controls and 63% in rats implanted with GH_3 cells). Thymidine incorporation at most mitogen doses was significantly greater in splenocytes from rats implanted with GH_3 cells than in control rats. Similarly, IL-2 synthesis was significantly augmented from splenocytes of GH_3-implanted rats (Table 1). Although GH_3 adenoma cells augmented both T-cell mitogenic activity and IL-2 synthesis in 24-month-old rats, neither of these responses was restored to levels observed in young rats. This effect may have occurred because thymic tissue had not been totally restored in the 24-month-old rats.

Lymphoid Cell Subpopulations. Although only thymic remnants could be detected in the control 24-month-old rats, we were able to prepare enough thymocytes from these rats for analysis by flow microfluorimetry. Wright-stained preparations revealed that these thymocytes consisted of >95% lymphocytes. Thymic tissue from GH_3-implanted rats contained moderately more lymphocytes of Thy-1.1 and helper-T phenotypes (Table 2). Cells from the spleen and bone marrow were gated so that only lymphocytes were included in the analysis. No major differences caused by implanting GH_3 cells were detected in any lymphoid cell population in either

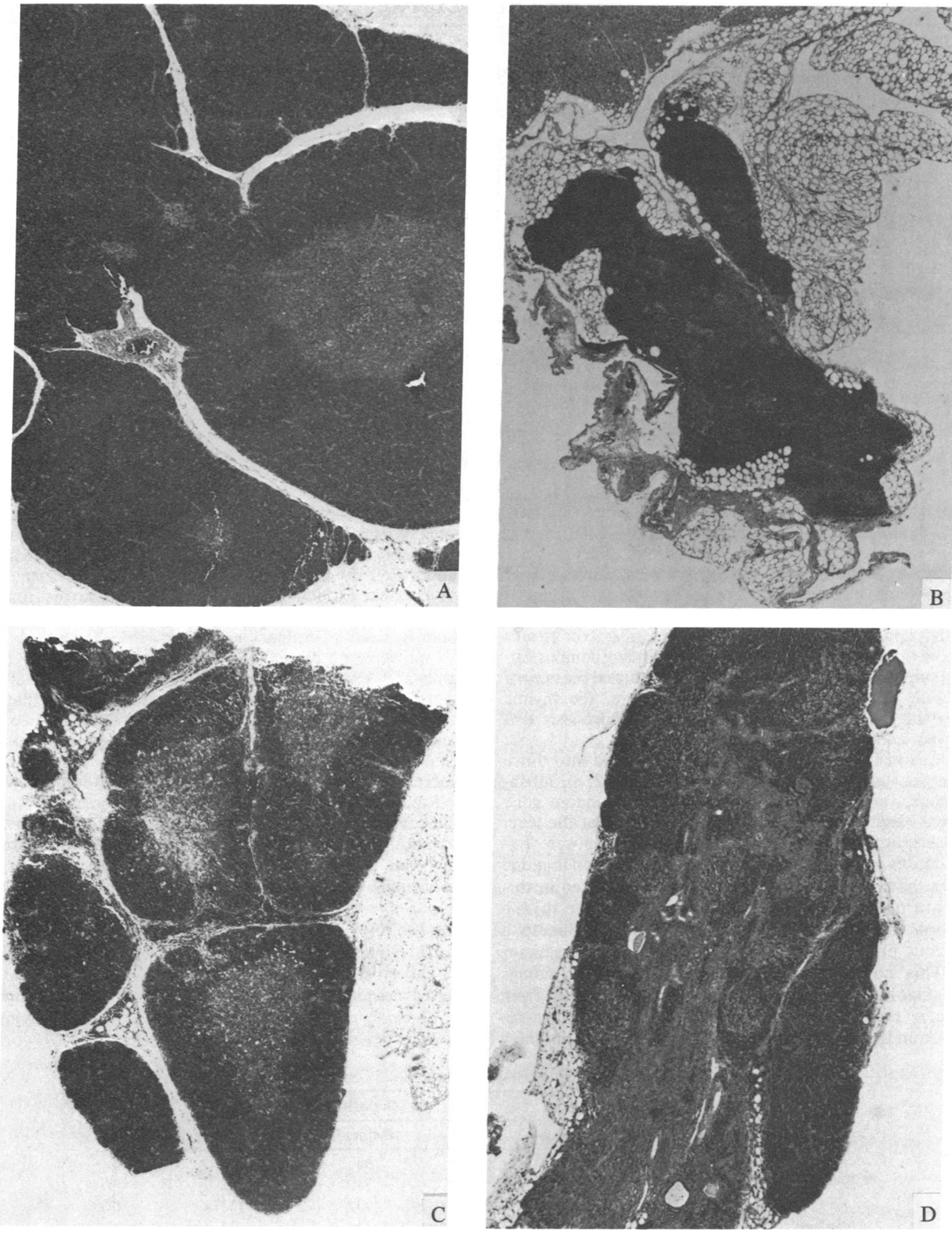

FIG. 1. Sections (4-μm) of thymus tissue from female Wistar-Furth rats. (*A*) Normal 3-month-old rat. (*B*) Twenty-four-month-old control rat. (*C*) Eighteen-month-old rat implanted 2 months earlier with GH_3 cells. (*D*) Twenty-four-month-old rat implanted 2 months earlier with GH_3 cells. (Hematoxylin and eosin; ×30.)

the spleen or bone marrow. The percentage of splenic T cells was unreasonably high, probably because the W3/13 monoclonal antibody recognized non-T cells. However, implanted GH_3 cells also did not affect the percentage of splenic helper or non-helper T cells, so it is unlikely that the GH_3 cells caused a major change in the proportion of splenic T cells.

DISCUSSION

We have shown that it is possible to restore normal thymic size and structure in 18-month-old Wistar-Furth rats. Thymic restoration was accompanied by significantly augmented proliferative responses to T-cell mitogens, and these responses

Table 1. Aged Wistar-Furth rats with GH_3 pituitary adenomas have augmented T-cell proliferation and IL-2 synthesis

Rats			Thymus weight,		Splenocyte [^{3}H]thymidine incorporation, cpm								IL-2 production, units	
					PHA (dilution)				Con A (μg/ml)				Control	
Age, months	GH_3*	*n*	% of body weight	Leukocytes, no. per μl	1:200	1:50	1:25	1:5	0.5	5	10	25	medium	Con A
3	−	6	0.07	4.0×10^3	850	6600	12,700	1700	32,100	4,500	2900	330	0	16
			(0.01)	(1.2×10^3)	(400)	(3000)	(5,500)	(900)	(17,700)	(1,500)	(1000)	(70)		(5)
18	−	4	UD	4.0×10^3	500	1500	2,500	200	3,000	2,600	1800	300	ND	ND
	+	4	0.05†	16.0×10^{3}†	1400‡	5800†	13,900†	300	6,800§	27,600‡	6600†	700‡	ND	ND
			(0.004)	(1.6×10^3)	(300)	(800)	(1,000)	(100)	(1,600)	(7,200)	(600)	(100)		
24	−	5	UD	3.9×10^3	200	200	300	200	200	200	200	200	0	0.4
	+	6	0.05†	12.1×10^{3}§	1500	2600†	1,900‡	1800‡	2,200	1,100†	700§	200	0	6.9‡
			(0.01)	(4.2×10^3)	(800)	(300)	(500)	(200)	(500)	(300)	(200)	(40)		2.2

Values in parentheses are standard errors. Only thymic remnants were detected in the control 18- and 24-month-old rats (UD, undefined), so zero was used for the statistical analysis. ND, not determined.
*GH_3 cells were injected (+) 2 months before the rats were killed.
†$P < 0.01$.
‡$P < 0.05$.
§$P < 0.10$.

were not different from those observed in 3-month-old rats. When 24-month-old rats were implanted with GH_3 cells, thymic structure was partially reconstituted, and this effect was accompanied by significant but incomplete restoration both of T-cell proliferative responses and of IL-2 synthesis. Further, it appeared that the proportions of T cells and T-cell subsets in rats with reconstituted thymus glands were similar to or greater than those proportions in control rats. This finding demonstrated that lymphoid cells in GH_3-implanted rats carried the normal distribution of phenotypic markers. Therefore, the thymic atrophy and the decline in T-lymphocyte blastogenesis that occur with aging are not irreversible.

Theories of immunologic aging can be divided into those revolving around intrinsic and extrinsic causes (2, 8). Intrinsic changes, such as preprogrammed aging, random gene mutations, and error-accumulating mechanisms at the level of transcription or translation have all been proposed. Extrinsic causes, such as neuroendocrine influences on immune function, have also been suggested. Data presented in this report are not in accord with the concept that the thymic involution that occurs during aging is entirely due to a genetically programmed destruction of thymic precursor cells. This conclusion is consistent with other findings (30–32) that have demonstrated that bone marrow cells from aged mice retain their full functional repertoire when removed from the aged environment. The present results show that whatever intrinsic changes occur in lymphoid cells from aged Wistar-Furth rats, these effects can be reversed by implanting GH_3 cells. It will therefore be important to understand the mechanisms by which GH_3 cells can augment thymus size and mitogenic capacity of splenocytes from aged rats. Likely candidates for this enhancement are extrinsic factors, such as pituitary hormones.

In a separate report (33), it was shown that body-weight gain and serum levels of insulin-like growth factor were significantly elevated in the GH_3-implanted rats that were used in the present experiments. This finding agrees with earlier studies (34, 35) in which GH_3 cells that were implanted into female Wistar-Furth rats caused a 20- to 100-fold increase in plasma growth hormone and a 10-fold elevation in plasma prolactin. Therefore, one of the simplest postulates is that the implanted pituitary adenoma cells augmented peripheral concentrations of growth hormone and/or prolactin in aged rats. The effects that we observed could have occurred by any of the following mechanisms. (*i*) The thymus gland in the GH_3-implanted animals may have processed progenitor T cells or residual thymocytes into functional T cells. However, because the percentage of splenic T cells and T-cell subsets was not augmented in GH_3-implanted rats, this effect could have occurred only if the spleen was repopulated with newly processed, functional T cells from the thymus. (*ii*) Newly developed thymic epithelial cells in the GH_3-implant-

Table 2. Distribution of lymphoid-cell subsets in 24-month-old control and GH_3-implanted rats

Tissue	Treatment	% positive cells				
		Thy-1.1	T	Helper-T	Non-helper T	Ia
Thymus	Control	61	80	64	50	30
	GH_3	77*	85	75*	62	30
		(7)	(6)	(5)	(11)	(9)
Spleen	Control	7	91	44	28	14
	GH_3	12†	82	50	36	15
		(3)	(7)	(8)	(6)	(5)
Bone marrow	Control	12	37	14	16	15
	GH_3	17	36	14	17	17
		(7)	(12)	(7)	(6)	(5)

Standard errors are given in parentheses. Percent positive cells using the secondary antibody in the absence of primary monoclonal antibody was 5% for thymic tissue, 10% for splenocytes, and 6% for bone marrow cells.
*$P < 0.05$.
†$P < 0.10$.

ed rats may have synthesized and secreted thymic hormones. These thymic hormones may have then permitted terminal differentiation of splenic T cells in peripheral lymphoid organs or directly enhanced their proliferative and IL-2-secreting capabilities. (*iii*) Hormones that were secreted by GH_3 cells may have directly affected lymphocytes. Lymphocytes have specific receptors for both growth hormone (36–38) and prolactin (39, 40), and both of these hormones affect functional activities of the immune system (18, 19, 41–47). Perhaps growth hormone, prolactin, or both acted directly on T lymphocytes to increase their ability to proliferate and secrete IL-2. All three of these possibilities are consistent with earlier findings in which T-cell-mediated immune events were restored in aged rats by syngeneic thymic grafts (5) or by treatment with either thymosin α_1 or IL-2 (6, 7, 26). Whatever the mechanism of action of GH_3 cells, these data show that factors extrinsic to the immune system can reverse some components of the decline in T-cell-mediated immunity that occurs during aging.

We gratefully acknowledge the helpful comments of Drs. H. Lewin, R. Dantzer, D. Segre, J. Shadduck, E. Voss, and J. Willis. This research was supported by Illinois Agriculture Experiment Station Project AS 20-0386, Moorman Manufacturing Company Grant 1-6-44620, and National Institutes of Health Grant AG06246-01.

1. Wade, A. W. & Szewczuk, M. R. (1984) *Adv. Immunol.* **36,** 143–188.
2. Makinodan, T. & Kay, M. (1980) *Adv. Immunol.* **29,** 287–330.
3. Weksler, M. E. (1980) *Proc. Soc. Exp. Biol. Med.* **165,** 200–205.
4. Makinodan, T. & Hirokawa, K. (1985) in *Relations Between Normal Aging and Disease*, ed. Johnson, H. A. (Raven, New York), pp. 117–132.
5. Hirokawa, K. & Makinodan, T. (1975) *J. Immunol.* **114,** 1659–1664.
6. Weksler, M. E., Innes, J. B. & Goldstein, G. (1978) *J. Exp. Med.* **148,** 996–1006.
7. Frasca, D., Garavini, M. & Doria, G. (1982) *Cell. Immunol.* **72,** 384–391.
8. Kay, M. (1984) *Mech. Ageing Dev.* **28,** 193–218.
9. Kay, M. (1979) *J. Invest. Dermatol.* **73,** 29–38.
10. Hayflick, L. (1965) *Exp. Cell Res.* **37,** 614–636.
11. Fabris, N. (1977) in *Comprehensive Immunology: Immunology and Aging*, eds. Makinodan, T. & Yunis, E. (Plenum, New York), pp. 73–89.
12. Price, G. B. & Makinodan, T. (1972) *J. Immunol.* **198,** 413–417.
13. Finkelstein, J. W., Roffwarg, H. R., Boyar, R. M., Kream, J. & Hellman, L. (1972) *J. Clin. Endocrinol.* **35,** 665–678.
14. Denkla, W. D. (1977) *Mech. Ageing Dev.* **6,** 143–152.
15. Gil-Ad, I., Gurewitz, R., Marcovici, O., Rosenfeld, J. & Laron, Z. (1984) *Mech. Ageing Dev.* **27,** 97–100.
16. Shrewsbury, M. M. & Reinhardt, W. O. (1959) *Endocrinology* **65,** 858–860.
17. Takemoto, H., Yokoro, K., Furth, J. & Cohen, A. I. (1962) *Cancer Res.* **22,** 917–924.
18. Pierpaoli, W., Baroni, C., Fabris, N. & Sorkin, E. (1969) *Immunology* **16,** 217–230.
19. Roth, J. A., Kaeberle, M. L., Grier, R. L., Hopper, J. G., Spiegel, H. E. & McAllister, H. A. (1984) *Am. J. Vet. Res.* **45,** 1151–1155.
20. Moloney, W. C., Boschetti, A. E. & King, V. (1969) *Cancer Res.* **29,** 938–946.
21. Tashjian, A. H., Jr., Yasumura, Y., Levine, L., Sato, G. H. & Parker, M. L. (1968) *Endocrinology* **82,** 342–352.
22. Boockfor, F. R., Hoeffler, J. P. & Frawley, L. S. (1985) *Endocrinology* **117,** 418–420.
23. Yamashita, S., Slanina, S., Kado, H. & Melmed, S. (1986) *Endocrinology* **118,** 915–918.
24. Leclerc, C., Morin, A., Deriaud, E. & Chedid, L. (1984) *J. Immunol.* **133,** 1996–2000.
25. Gillis, S., Kozak, R., Durante, M. & Weksler, M. E. (1981) *J. Clin. Invest.* **67,** 937–942.
26. Chang, M. P., Makinodan, T., Peterson, J. W. & Strehler, B. L. (1982) *J. Immunol.* **129,** 2426–2430.
27. Thoman, M. & Weigle, W. (1982) *J. Immunol.* **128,** 2358–2361.
28. Gilman, S. C., Rosenberg, J. S. & Feldman, J. D. (1982) *J. Immunol.* **128,** 644–650.
29. Rosenberg, J. S., Gilman, S. C. & Feldman, J. D. (1983) *J. Immunol.* **130,** 1754–1758.
30. Gorczynski, R. M., Chang, M., Kennedy, M., MacRae, S., Benzing, K. & Price, G. B. (1984) *Immunopharmacology* **7,** 179–194.
31. Goidl, E., Choy, J. W., Gibbons, J. J., Weksler, M. E., Thorbecke, G. L. & Siskind, G. W. (1983) *J. Exp. Med.* **157,** 1635–1645.
32. Astle, C. M. & Harrison, D. E. (1984) *J. Immunol.* **132,** 673–677.
33. Turner, J. D., Novakofski, J. & Bechtel, P. J. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45,** 402 (abstr. 1441).
34. Prysor-Jones, R. A. & Jenkins, J. S. (1980) *J. Endocrinol.* **85,** 75–82.
35. Seo, H., Refetoff, S. & Fong, V. S. (1977) *Endocrinology* **100,** 216–226.
36. Arrenbrecht, S. (1974) *Nature (London)* **252,** 255–257.
37. Lesniak, M. A., Gorden, P., Roth, J. & Gavin, J. R., III (1974) *J. Biol. Chem.* **249,** 1661–1667.
38. Kiess, W. & Butenandt, O. (1985) *J. Clin. Endocrinol. Metab.* **60,** 740–746.
39. Russell, D. H., Kibler, R., Matrisian, L., Larson, D. F., Poulos, B. & Magun, B. E. (1985) *J. Immunol.* **134,** 3027–3031.
40. Russell, D. H., Matrisian, L., Kibler, R., Larson, D. F., Poulos, B. & Magun, B. E. (1984) *Biochem. Biophys. Res. Commun.* **121,** 899–906.
41. Gisler, R. H. & Schenkel-Hulliger, L. (1971) *Cell. Immunol.* **2,** 646–657.
42. Pandian, M. R. & Talwar, G. P. (1971) *J. Exp. Med.* **134,** 1095–1113.
43. Duquesnoy, R. J. (1972) *J. Immunol.* **108,** 1578–1590.
44. Nagy, E., Berczi, I. & Friesen, H. G. (1981) *Allergy* **36,** 429–431.
45. Nagy, E., Berczi, I. & Friesen, H. G. (1983) *Acta Endocrinol.* **102,** 351–357.
46. Nagy, E., Berczi, I., Wren, G. E., Asa, S. L. & Kovacs, K. (1983) *Immunopharmacology* **6,** 231–243.
47. Snow, E. C. (1985) *J. Immunol.* **135,** 776s–778s.