American Journal of Pathology, Vol. 160, No. 6, June 2002

Life Span Extension by Reduction in Growth Hormone-Insulin-Like Growth Factor-1 Axis in a Transgenic Rat Model

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The longer life span in dwarf mice suggests that a reduction in the growth hormone (GH)-insulin-like growth factor (IGF)-1 axis retards aging and extends the life span in mammals. We tested this hypothesis in a transgenic strain of rats whose GH gene was suppressed by an anti-sense GH transgene. Male rats homozygous for the transgene (tg/tg) had a reduced number of pituitary GH cells, a lower plasma concentration of IGF-1, and a dwarf phenotype. Heterozygous rats (tg/−) had an intermediate phenotype in plasma IGF-1, food intake, and body weight between tg/tg and control (−/−) rats. The life span of tg/tg rats was 5 to 10% shorter than −/− rats. In contrast, the life span of tg/− rats was 7 to 10% longer than −/− rats. Pathological analysis suggested that neoplasms caused earlier death in tg/tg rats; in contrast, tg/− rats had reduced nonneoplastic diseases and a prolonged life span. Immunological analysis revealed a smaller population and lower activity of splenic natural killer cells in tg/tg rats. The results of the present study support the hypothesis, but suggest that there is an optimal level of the GH-IGF-1 axis to maximize survival in mammals. ***(Am J Pathol 2002, 160:2259–2265)***

Longevity-associated genes have been isolated in lower organisms such as yeasts, flies, and nematodes.[1] In mammals, *Prop-1* was the first gene reported to be associated with a longer life span.[2] Ames dwarf mice, in which a homozygous mutation of the *Prop-1* gene leads to near absence of growth hormone (GH)-, prolactin-, and thyroid-stimulating hormone-producing cells in the pituitary gland, live much longer than their normal counterparts. The phenotypically similar Snell dwarf mice with a mutation of the *pit-1* gene also has a longer life span.[3] These reports do not exclude a potential life span-extending effect of prolactin or thyroid-stimulating hormone or their combination. Inhibition of GH-dependent pathways, however, is thought to have a principal role in life span extension, because several premature aging phenomena are observed in GH-overexpressing mice.[4]

Using a transgenic strain of rats in which GH synthesis and release were reduced by overexpression of the anti-sense GH transgene, we conducted a longevity study to elucidate whether isolated suppression of GH and the downstream pathways retard the aging process and extend the life span. During the study, it was reported that GH receptor/binding protein (GHR/BP) gene-disrupted mice and "little" mice with a mutation of the GH-releasing hormone receptor gene also have a longer life span,[3,5] providing additional evidence of a substantial role for the GH-IGF-1 axis in life span.

The present study provided data of life span, pathology, and immunological parameters of GH-insulin-like growth factor (IGF)-1-suppressed transgenic rats.

Materials and Methods

Rats and Husbandry

The transgenic male rats (mini, Jcl:Wistar-TgN(ARGHGEN)1Nts) used in the present study were kindly provided by Nippon Institute for Biological Science (Oume City, Tokyo, Japan), where the closed colony of homozygous mini rats was established and maintained. The mini rats were produced from founders created by introducing a fusion gene into rat embryos.[6] The genetic background was Jcl:Wistar (Japan Clea, Inc., Tokyo, Japan). The transgene consisted of four copies of thyroid hormone response elements, rat GH promoter, and anti-sense cDNA sequences for rat GH. Transgenic offspring expressed the rat GH anti-sense transgene in the pituitary gland, exhibited dwarfism as early as 3 weeks of age, and a

Supported in part by the Research Grant for Longevity Sciences (grant 11-C) from the Ministry of Health, Welfare, and Labor of Japan.

Accepted for publication March 18, 2002.

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reduced growth rate thereafter. Reproductive function was, however, almost normal in both male and female mini rats, although the fecundity in female mini rats was slightly lower than in control Wistar female rats. F1 hybrid rats (Jcl:Wistar-TgN(ARGHGEN)1Nts × Jcl:Wistar) were also generated at our laboratory animal center to moderate the reduced plasma concentrations of GH and IGF-1. Female Wistar rats (Jcl:Wistar, Japan Clea, Inc.) were mated with male mini rats. Control male rats (Jcl:Wistar) were purchased from Japan Clea, Inc. All male rats used in the present study were born between February 17 and February 27, 1998. In the present study, the three groups of rats were referred to as tg/tg, tg/−, and −/− rats.

At 4 weeks of age, weanling male rats of the three groups were transferred to a barrier facility (temperature, 24°C ± 1°C; 12-hour light/dark cycle), housed separately and maintained under specific pathogen-free conditions during the present experiment. Rats were provided with CR-LPF diet (Oriental Yeast Co. Ltd., Tsukuba, Japan), which is based on the formula of Charles River Inc. (CRF-1), but the fraction of protein is reduced by 18% for the long-term study. The composition of the diet is as follows (per 100 g); 18.2 g protein, 4.8 g fat, 6.6 g mineral mixture, 5.0 g fiber, 57.9 g nitrogen-free water-soluble substance, 7.5 g water. The caloric value of the diet is 348 kcal/100 g. All rats were fed the diet and water *ad libitum*.

There were two sets of rats: a longevity group and a cross-sectional group. Rats in the longevity group (n = 30) were maintained until spontaneous death. Rats in the cross-sectional group (n = 12 to 16) were killed at 6-, 15-, and 24-months of age to collect tissues and measure biomarkers (n = 5 to 10 for each rat group at each age).

The amount of diet consumed by each rat group was measured at 1-week intervals until 12 weeks of age, at 2-week intervals until 24 weeks of age, and thereafter at 4-week intervals. Ten rats in each group were selected for regular measurement. Body weight of each rat was measured at 2-week intervals until 24 weeks of age, and thereafter at 4-week intervals.

The specific pathogen-free (SPF) status of the rat colony was monitored on receipt of the rats and every 6 months thereafter by serological examination for microorganisms in sera from sentinel rats. Antibodies of Sendai virus, sialodacryoadenitis virus, *Mycoplasma pulmonis*, *Clostridium piliforme*, hantavirus, H-1 virus, Kilham rat virus, minute mouse virus, mouse encephalomyelitis virus, mouse adenovirus, pneumonia mouse virus, reovirus type 3, and CAR bacillus were negative as determined by enzyme-linked immunosorbent assay or indirect immunofluorescence methods during the present experiment.

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation at our institution.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was prepared from pituitary, spleen, thymus, lung, liver, heart, kidney, and testis tissues in rats at 6 months by the guanidinium thiocyanate/acid-phenol-chloroform method using the Isogen kit (Nippon Gene Inc., Toyama, Japan). According to the protocol provided by the manufacturer, the RT reaction was performed using GeneAmp RNA PCR Kit (Perkin Elmer, Norwalk, CT). Primers specific for the transgene were made according to the original article;[6] forward: 5′-AGAATCCA-GATGCTCAAGGCCC-3′, reverse: 5′-AGCCATCGCCAC-TCAGTGATCT-3′. The amplified products were 319 bp, and included the sequence between a part of the antisense rat GH cDNA and the upstream region of the human β-globin poly-(A) site. PCR was performed under the following conditions: 2 minutes at 94°C for the initial denaturation, then 30 seconds at 94°C, 30 seconds at 60°C, 1 minute at 72°C for 20 to 30 cycles, followed by a 7-minute extension at 72°C. The PCR products were electrophoresed on a 3% Nusieve 3:1 agarose (BioWhittaker Molecular Applications Inc., Vallensbaek Strand, Denmark) gel and stained with SYBR' Gold Nucleic Acid Gel Stain (Molecular Probes Inc., Eugene, Oregon). After destaining, SYBR' Gold luminescence was acquired using FLA-3000 (Fuji Film Co., Ltd., Tokyo, Japan).

Immunohistochemistry

Immunohistochemical examination for pituitary hormones was performed in formalin-fixed, paraffin sections of pituitary glands removed from rats at 6 months of age as previously described.[7] The primary antibodies used were: antibody to rat GH (Shikibo, Inc., Kurashiki, Japan), antibody to prolactin (Biogenesis, Ltd., Poole, UK), antibody to thyroid-stimulating hormone (Biogenesis, Ltd.), antibody to adrenocorticotropic hormone (Ylem SRL, Avenzazano, Italy), antibody to luteinizing hormone (DAKO Corp., Carpinteria, CA), antibody to follicle-stimulating hormone (DAKO Corp., Carpinteria, CA). The avidin-biotin-peroxidase complex (ABC) method was used to visualize the pituitary hormones. Biotinylated secondary antibodies and the ABC kit were purchased from Vector Laboratories, Inc. (Burlingame, CA). The substrate for peroxidase was 3,3′-diaminobenzidine-tetrahydrochloride with 0.001% H_2O_2, which yielded brown reaction products. Negative control was achieved by replacing the primary antibodies with nonimmunized mouse or rabbit serum samples.

Plasma Concentrations of GH and IGF-1

Plasma samples were prepared from trunk blood after decapitation and stored at −30°C until performing enzyme-immunoassay or radioimmunoassay. The concentration of GH was measured using enzyme-immunoassay kits (Amersham Pharmacia Biotech, Little Chalfont, UK). The concentration of IGF-1 was measured using radioimmunoassay kits (Nichols Institute Diagnostics, San Juan Capistrano, CA). The samples for IGF-1-radioimmunoassay were acidified and extracted, according to the instructions provided by the manufacturer. The intra-assay and interassay coefficients of variation for enzyme-immunoassay and radioimmunoassay were less than 10%, respectively.

Pathological Examination

All rats were inspected at least once daily. Dead rats were removed from the cage and either autopsied immediately or refrigerated for a brief period. Autolysis was almost never severe enough to prevent histopathological examination. The following organs or tissues were excised, fixed in 10% formalin, and routinely processed for histopathological examination: brain, pituitary gland, heart, lung, trachea, aorta, esophagus, stomach, small intestine, liver, pancreas, spleen, kidney, urinary bladder, prostate, testis, epididymis, thyroid gland, adrenal gland, femoral muscle, femoral bone, sternum, skin, and eye. Probable cause of death was determined after autopsy. Anterior pituitary adenoma and chronic nephropathy, potentially lethal diseases, are prevalent in aging rats.[8–10] Therefore, the prevalence of these diseases was also examined. The severity of chronic nephropathy was scored using the method of Maeda and colleagues[8] with some modifications (Table 3).

Immunological Parameters

The procedures for flow cytometry, natural killer (NK) activity, and determination of the mitogenic response to stimulators was previously described.[9,10] Briefly, cell suspensions prepared from spleen and thymus were first treated with 0.83% NH_4Cl to remove red blood cells and washed with RPMI 1640 containing 2% fetal bovine serum. The cell suspension was stained with monoclonal antibodies, and then assessed using a FACScan (Becton Dickinson, Mountain View, CA). The lymphocyte fraction was gated using forward and side scatter signals to exclude myeloid cells. The percentage of lymphocyte subsets was analyzed using CELLQuest software (Becton Dickinson, Mountain View, CA). Monoclonal antibodies used were anti-CD3 (clone G4.18), anti-CD45R (clone HIS24), anti-NKR-P1A (clone 10/78), anti-CD4 (clone OX-38), and anti-CD8 (clone OX-8). All antibodies were purchased from PharMingen (San Diego, CA). The anti-CD3 and anti-CD8 antibodies were labeled with fluorescein isothiocyanate; the other antibodies were labeled with phycoerythrin. Splenic lymphocyte subsets were defined by subsequent two-color staining: T cells (CD3+/CD45R−), B cells (CD3−/CD45R+), and NK cells (CD3−/NKR+). Thymocyte subpopulations were evaluated by combining CD4 and CD8 staining.

To assess the NK cell activity in splenic cells, a fixed number of ^{51}Cr-labeled YAC-1 target cells (4×10^4) was mixed with either 0.5, 1, 2, or 4×10^6 spleen cells in a total volume of 0.2 ml in a microplate with round-bottomed wells. The plate was incubated for 5 hours, centrifuged, and the radioactivity of the supernatant was counted by a γ-counter (ARC-380; Aloka Co. Ltd., Tokyo, Japan).

Mitogenic responses of splenic cells were also evaluated. Assays were performed in 96-well flat-bottom microplates (Falcon no. 3072; Becton Dickinson Labware, Franklin Lake, NJ). Spleen cells (5×10^5) in 0.2 ml of RPMI 1640 medium, supplemented with 5% fetal bovine serum, were stimulated with an optimum dose of phytohemagglutinin, concanavalin A (ConA), and anti-CD3 mAb (145-2C11; hereafter referred to as anti-CD3; 1.0 μg/ml; PharMingen, San Diego, CA). The plates were incubated at 37°C in 5% CO_2 in air atmosphere. After appropriate intervals, 9.25 kBq of [^{3}H]thymidine in 5 μl was added, and 2 hours later the cells were harvested and processed for β-scintillation counting (MicroBeta 1450; Pharmacia Biotech, Turku, Finland).

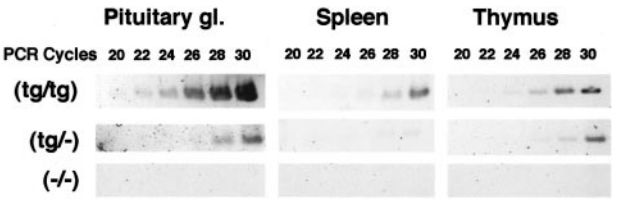

Figure 1. Transgene expression detected by RT-PCR in the pituitary gland, spleen, and thymus at 6 months of age.

Statistical Analysis

The plasma concentrations of GH and IGF-1 and the immunological parameters were analyzed using one-way analysis of variance and Fisher's protected least significant difference (PLSD) post hoc test for multiple comparisons. The survival curves were estimated using Kaplan-Meier's estimates, and curves were compared using the log-rank test. The summarized frequency of causes of death (neoplastic and nonneoplastic), the prevalence of pituitary adenoma, and the prevalence of chronic nephropathy were analyzed using a chi-square test or Fisher's exact test. All statistical analyses were performed using StatView 5.0 software (SAS Institute Inc., Cary, NC). A p value of 0.05 was considered to be statistically significant.

Results

Expression of Anti-Sense GH Gene and Immunohistochemistry in the Pituitary Gland

The anti-sense GH-mRNA expression was detected by RT-PCR in the pituitary, spleen, and thymus in tg/tg and tg/− rats; there was no anti-sense GH-mRNA expression in those organs in control −/− rats (Figure 1). The amount of amplified products was greater in tg/tg rats than in tg/− rats. There were no amplified products in samples from the lung, liver, heart, kidney, and testis in tg/tg and tg/− rats (data not shown).

Immunohistochemistry revealed that there were fewer GH-positive cells in the pituitary gland in tg/tg rats than in tg/− and −/− rats (Figure 2). There were also fewer GH cells in tg/− than in −/− rats. The other pituitary hormones were expressed similarly in the three rat groups (data not shown).

Plasma Concentrations of GH and IGF-1

The plasma concentration of GH did not statistically differ among the three rat groups (Table 1). The plasma con-

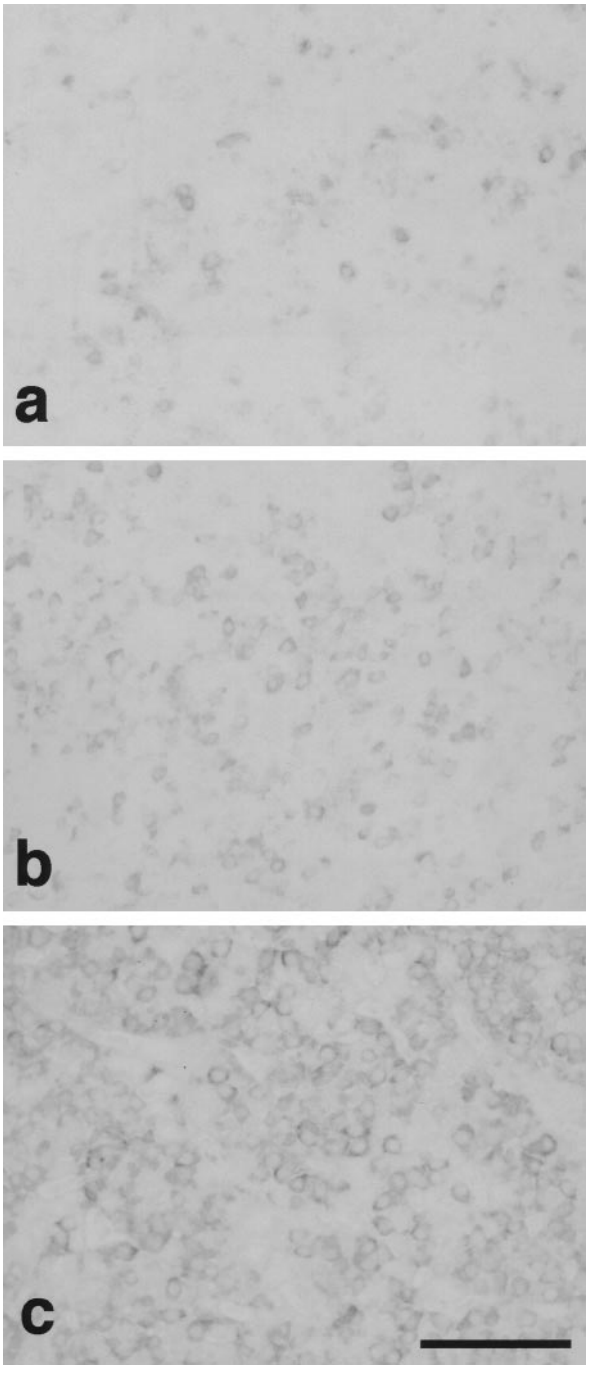

Figure 2. Immunohistochemistry for GH in the pituitary gland at 6 months of age: **a**, tg/tg; **b**, tg/−; **c**, −/−. Scale bar, 100 μm (**c**).

centrations of IGF-1 were decreased by 74% in tg/tg rats and by 39% in tg/− rats, as compared with that in −/− rats.

Food Intake and Body Weight

The food intake in −/− rats did not change appreciably with increasing age after 12 weeks, although it was slightly reduced after 108 weeks (Figure 3). The food intake pattern with aging in tg/tg and tg/− rats was similar to that in −/− rats. The tg/tg rats and tg/− rats consumed ~50% and 70 to 80% of the mean intake of −/− rats, respectively.

Table 1. Plasma Concentrations of GH and IGF-1 in Rats at 6 Months of Age

	tg/tg	tg/−	−/−
GH (ng/ml)	142.8 ± 33.9	172.1 ± 44.1	157.3 ± 55.0
IGF-1 (ng/ml)	255.8 ± 21.4*	603.0 ± 86.3†	983.7 ± 130.4

Values represent the mean ± SD (n = 5 to 8).
*tg/tg *versus* tg/−, $P < 0.0001$, tg/tg *versus* −/−, $P < 0.0001$.
†tg/− *versus* −/−, $P < 0.0001$.

The −/− rats gained weight until 96 weeks of age, then, gradually lost weight (Figure 4). The body weight pattern throughout the life span was similar in tg/tg and tg/− rats. The weight in tg/tg rats was 41 to 45% of that in −/− rats for most of the experimental period. The weight in tg/− rats was 66% of that in −/− rats at 24 weeks of age, and gradually increased until reaching 76% of the weight in −/− rats at 96 weeks of age.

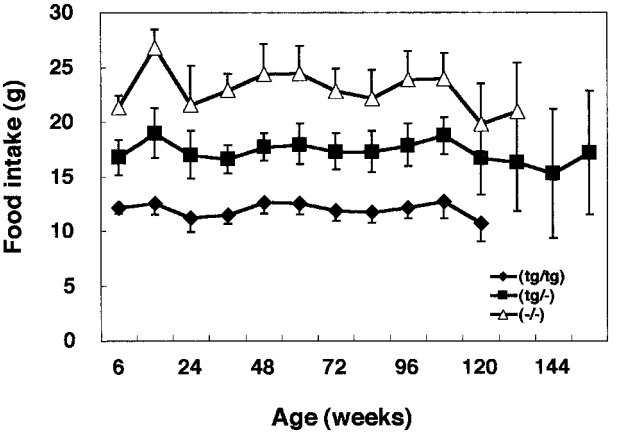

Figure 3. Food intake (g/rat/day) in rat groups in the longevity study. The points represent the mean ± SD (n = 10). Data of 12-week intervals are shown, and are not depicted when the number of rats was below five.

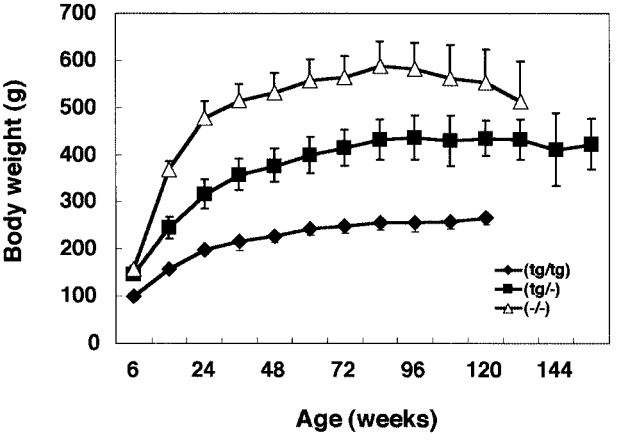

Figure 4. Body weight in rat groups of the longevity study. The points represent the mean ± SD; n = 30 for each group at the start of the study decreased with age. Data are not depicted when the number of rats was below five.

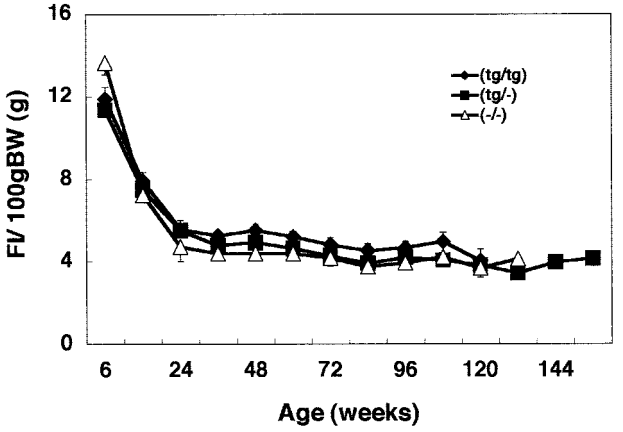

Figure 5. Food intake/100 g body weight (FI/100gBW) in rat groups of the longevity study. The points represent the mean ± SD (n = 10). Data of 12-week intervals are shown, and are not depicted when the number of rats was below five.

Food intake, if normalized by body weight, was decreased precipitously between 6 and 24 weeks; thereafter, it decreased only slightly (Figure 5). At 6 weeks of age, the food intake/100 g body weight (FI/100gBW) was less in tg/tg and tg/− rats than −/− rats. There was no difference between tg/tg and tg/− rats. Between 24 and 120 weeks, however, FI/100gBW was 17.5% and 8.7% greater in tg/tg rats and tg/− rats, as compared to −/− rats.

Longevity Data

The survival curve of tg/tg rats differed significantly from that of control −/− rats ($P < 0.01$, Figure 6). The life span decreased by 7 weeks (5%, 50th percentile) and 14 weeks (10%, 25th percentile) in tg/tg rats, as compared with that in −/− rats (Table 2). The survival curve of tg/− rats also differed from that of −/− rats ($P = 0.0323$). The life span was greater by 12 weeks (7%, 50th percentile) and 14 weeks (10%, 25th percentile) in tg/− rats, as compared with that in −/− rats. The maximum life span was the following: 149 weeks tg/tg, 171 weeks tg/−, and 158 weeks −/−, ie, 6% less in tg/tg rats and 8% greater in tg/− rats, as compared to −/− rats.

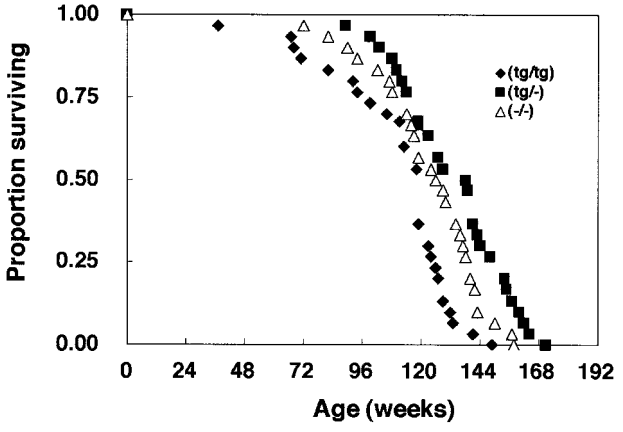

Figure 6. Group survival curves (n = 30 at the start of the study).

Table 2. Summary of Life Span Data

	tg/tg	tg/−	−/−
Age of 50th percentile	119 ± 2	138 ± 14	126 ± 7
Age of 25th percentile	126 ± 3	154 ± 5	140 ± 3
Maximum length of life	148	171	158

Values represent estimated values ± SD (week) for the ages of 50th and 25th percentile.

Pathological Parameters

Probable causes of death differed among the three rat groups, when data were summarized into two categories, neoplastic and nonneoplastic causes. The tg/tg rats died mostly of neoplasms, particularly leukemia, which was never observed in −/− rats (Table 3). The tg/− rats died of neoplasms; the proportion of nonneoplastic causes was also increased as compared to tg/tg rats. In −/− rats, the proportion of nonneoplastic causes of death was further increased as compared to tg/− rats, although this difference was not statistically significant ($P = 0.0631$).

The prevalence of pituitary adenoma was significantly decreased in tg/tg rats; there was no difference between tg/− and −/− rats (Table 4). There was no kidney lesions in tg/tg rats. The prevalence of chronic nephropathy was significantly decreased in tg/− rats than in −/− rats.

Immunological Parameters

Spleen weights were lower in the following order; tg/tg, tg/−, −/− rats [401 ± 28 in tg/tg, 632 ± 42 in tg/−, and 913 ± 172 in −/− rats; mean ± SD (mg) of five to six rats]. Thymus weights were lower in tg/tg rats; there was no statistical difference between tg/− and −/− rats [92 ± 17 in tg/tg, 162 ± 46 in tg/−, and 194 ± 41 in −/−; mean ± SD (mg) of five to six rats]. When normalized by body weight, spleen and thymus weights did not differ significantly among the three groups (data not shown).

The proportions of T cells (CD3+/CD45R−) and B cells (CD3−/CD45R+), and thereby the T/B cell ratio in the spleen did not differ significantly among groups (Ta-

Table 3. Probable Causes of Death

Lesions	Rats		
	tg/tg	tg/−	−/−
Nonneoplastic	1/30	8/30	15/30
Nephropathy	0	0	2
Cardiac thrombus	0	3	6
Others/undetermined	1	5	7
Neoplastic	29/30*	22/30†	15/30
Leukemia	16	2	0
Pituitary tumor	0	11	5
Others	13	9	10

Fractions represent the number of rats that died of nonneoplastic or neoplastic causes of death/the number of rats examined. Frequency of specific causes are also presented.

*$P < 0.05$ *versus* tg/− and −/−; †$P = 0.0631$ *versus* −/− by Fisher's exact test.

Table 4. Prevalence of Anterior Pituitary Adenoma and Chronic Nephropathy in Rats that Died Spontaneously

Lesions	Rats		
	tg/tg	tg/−	−/−
Pituitary adenoma	7/30*	22/30	20/30
Chronic nephropathy	0/30*	8/30†	25/30
Mild	0	7	17
Moderate	0	1	5
Severe	0	0	3

Fractions represent the number of rats with pituitary adenoma or chronic nephropathy/the number of rats examined.
*$P < 0.05$ *versus* tg/− and −/−.
†$P < 0.05$ *versus* −/−. Frequency of rats with nephropathy by severity, mild, moderate, and severe, which correspond grade 1 to 2, grade 3, and grade 4-E of Maeda et al.,[8] are also presented. Details of the system for grading chronic nephropathy are described by Maeda et al.[8]

ble 5). The proportion of NK cells (NKR+/CD3−) was smaller in tg/tg rats, as compared to −/− rats; There was no difference between tg/− and −/− rats.

The mitogenic response of splenic T cells, examined by the response to phytohemagglutinin, Con A, and anti-CD3 antibodies, did not differ significantly among groups (data not shown). NK activity was significantly lower in tg/tg rats than in tg/− and −/− rats; NK activity in tg/− rats did not differ from that in −/− rats (Table 5). The proportion of thymocyte subsets did not differ significantly among groups (data not shown).

Discussion

This transgenic model is suitable for evaluating the relation between the GH-IGF-1 axis and longevity in animals, because of the strategically direct method of inducing an isolated suppression of the GH-IGF-1 axis. Although we did not evaluate the pulsatile profile of GH secretion and therefore did not demonstrate direct evidence of reduced GH secretion, immunohistochemical stains for pituitary hormones, the plasma concentration of IGF-1, and the dwarf phenotype indicated a reduction in the GH-IGF-1 axis. Another important aspect of the present study was the generation of rats heterozygous for the transgene. The tg/− rats exhibited an intermediate phenotype in food intake, body weight, and plasma IGF-1. The present study evaluated the effects of a reduced GH-IGF-1 axis on life span, pathology, and immunological parameters at severe and moderate levels of suppression.

Table 5. Immunological Parameters in Rats at 6 Months of Age

Parameters	Rats		
	tg/tg	tg/−	−/−
Lymphocyte subpopulation in splenic cells			
CD3+ (%)	40.8 ± 4.2	44.3 ± 5.4	40.5 ± 9.3
CD45R+ (%)	39.2 ± 3.4	42.7 ± 5.1	45.1 ± 9.0
T/B cell ratio	1.06 ± 0.20	1.06 ± 0.23	0.97 ± 0.48
NKR+/CD3− (%)	5.77 ± 0.59*	6.99 ± 1.30	7.50 ± 1.49
NK cell activity (%) in splenic cells			
	23.3 ± 5.1†	33.2 ± 6.6	37.8 ± 8.0

Values represent the mean ± SD ($n = 5$ to 6).
*$P < 0.05$ *versus* −/−.
†$P < 0.05$ *versus* tg/− and −/−.

The present study demonstrated that suppression of GH-IGF-1 axis extended the life span in rats. This finding, however, differed from those previously reported in specific strains of mice in the following two points. First, a severe reduction in the GH-IGF-1 shortened the life span in rats. Second, life span was extended by only 10%, which is considerably less than that in the mice models.

In the dwarf mice models,[2,3] the hormonal effects of GH and IGF-1 in tissues are markedly reduced. *Prop-1* or *Pit-1* gene mutation leads to near absence of GH. GHR/BP-disruption induces very low concentrations of plasma IGF-1.[5] In this model, plasma GH concentrations are increased because of negative feedback mechanisms; however, the GH signaling pathway is completely disrupted. Nonetheless, the life span is extended in all dwarf mice models. The present pathological analysis suggests that neoplasms, particularly leukemia, caused earlier death in tg/tg rats. During the course of establishment of the closed colony of the transgenic rats, specific alleles for leukemia might be selected. Other types of neoplasms, however, also caused earlier death in tg/tg rats, suggesting that a severely reduced GH-IGF-1 axis promotes tumorigenesis. Although many aspects of tumorigenesis should be investigated, the present analysis of the immune system provides possible causes for the promotion of tumorigenesis. The NK cell activity and population decreased in tg/tg rats. GH and IGF-1 are required for normal development of the immune system,[11] which intrinsically prevents tumorigenesis in animals.[12] Therefore, the severely reduced GH-IGF-1 axis might promote tumorigenesis by reducing immune function, particularly NK cell activity. In this regard, however, Snell mice, in which the GH-IGF-1 axis is more severely depressed than in tg/tg rats, achieve normal immune responsiveness, although they lag behind their heterozygous littermates for development of immunocompetence.[13] Age-dependent declines in T-lymphocyte subsets and functions are also blunted in Snell mice.[3] We should investigate a possible difference in the development of the immune system in response to GH and IGF-1 between the present rat model and dwarf mice, thus, implicating involvement of the immune system in tumorigenesis in the rodent models.

Although tg/− rats have a longer life expectancy, it is not known whether the aging process is retarded by moderate suppression of GH-IGF-1 axis in rats. The life-prolonging effect in rats observed in the present study was 10%, whereas it is 33% in female Ames, 40% in male Ames,[2] and 42% in Snell mice.[3] Caloric restriction, a well-known intervention that retards aging processes and delays or prevents disease processes, increases median life span by 28.2% in male F344 rats.[14] Replacement of casein with soy protein as a protein source in the diet extended the median life span by 15.6%, even though caloric intake was not restricted.[11] Pathological examination of these rats revealed that this life span extension could be mostly because of the prevention of chronic nephropathy, but not to retardation of the aging process

per se.[11] In other words, life span extension is expected to some extent if an intervention retards or prevent a few potentially lethal diseases. The present pathological findings also suggest that nonneoplastic diseases, including cardiac thrombus and chronic nephropathy, could be prevented in tg/− rats, whereas there might be little effect on the onset and progression of pituitary adenoma. It is possible that the reduced incidence of lethal nonneoplastic diseases slightly extends life span and therefore pituitary adenoma increases in tg/− rats as a cause of death, although the onset and progression of pituitary tumor is not altered in tg/− rats, as compared with −/− rats. Another explanation is that the onset rate is similar, but the progression of pituitary adenoma is accelerated in tg/− as compared to −/− rats. Because of the limited number of rats examined, we could not analyze the effects of GH-IGF-1 axis on prevalence of nonneoplastic diseases such as cardiac thrombosis. The prevalence of chronic nephropathy, from which laboratory rats often suffer during long-term life-span study,[8] however, was clearly reduced by suppression of the GH-IGF-1 axis. This finding is consistent with previous findings in that GH or IGF-1 promotes experimentally induced diabetic and nondiabetic nephropathy in rodents.[15,16]

Bartke and colleagues[17] suggested that resistance of dwarf mice to oxidative and glycative stresses contribute to extend life span. Further studies on biomarkers of aging are needed to evaluate the life-prolonging effect of moderate suppression of the GH-IGF-1 axis in rats; however the reduced GH-IGF-1 could extend the life span in rats, if not retard the physiological aging process.

An age-related decline in plasma concentrations of GH and IGF-1 induces several aging phenotypes such as reduced bone mass and increased fat tissues in aging animals Replacement therapy of these anabolic hormones restores these phenotypes, at least in part.[18] Regarding longevity in animals, however, most studies of experimental models, including those in lower organisms, indicate that a reduction in the GH-IGF-1 axis or related signal pathways induce life span extension.[1,19] To our knowledge, only one study reports that long-term, low-dose administration of GH in mice in later life extends life expectancy.[20] On the other hand, overexpression of GH in mice promotes some of the aging processes and shortens life span.[4] The findings of the present study suggest that there could be an optimal level of the GH-IGF-1 axis to maximize survival in organisms, ie, the axis is required for normal development of intrinsic physiological systems such as the immune system in an early stage of life, but not to the extent that the axis promotes disease and the aging process. Further studies on the influence of the GH-IGF-1 axis in aging phenotypes and longevity will provide insight to better understand the aging process in a variety of organisms.

Acknowledgment

We thank Yutaka Araki and the staff in the laboratory animal center at Nagasaki University School of Medicine for their excellent technical support.

References

1. Guarente L, Kenyon C: Genetic pathways that regulate ageing in model organisms. Nature 2000, 408:255–262
2. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A: Dwarf mice and the aging process. Nature 1996, 384:33
3. Flurkey K, Papaconstantinou J, Miller RA, Harrison DE: Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proc Natl Acad Sci USA 2001, 98:6736–6741
4. Steger RW, Bartke A, Cecim M: Premature ageing in transgenic mice expressing different growth hormone genes. J Reprod Fertil 1993, 46:S61–S75
5. Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ: Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology 2000, 141:2608–2613
6. Matsumoto K, Kakidani H, Takahashi A, Nakagata N, Anzai M, Matsuzaki Y, Takahashi Y, Miyata K, Utsumi K, Iritani A: Growth retardation in rats whose growth hormone gene expression was suppressed by antisense RNA transgene. Mol Reprod Dev 1993, 36:53–58
7. Shimokawa I, Tomita M, Higami Y, Okimoto T, Kawahara T, Ikeda T: Dietary restriction maintains the basal rate of somatotrope renewal in later life in male rats. Age 1997, 20:169–174
8. Maeda H, Gleiser CA, Masoro EJ, Murata I, McMahan CA, Yu BP: Nutritional influences on aging of Fischer 344 rats: II. Pathology. J Gerontol 1985, 40:671–688
9. Ishiyama N, Utsuyama M, Kitagawa M, Hirokawa K: Immunological enhancement with a low dose of cyclophosphamide in aged mice. Mech Ageing Dev 1999, 111:1–12
10. Utsuyama M, Seidlar H, Kitagawa M, Hirokawa K: Immunological restoration and anti-tumor effect by Japanese herbal medicine in aged mice. Mech Ageing Dev 2001, 122:341–352
11. Iwasaki K, Gleiser CA, Masoro EJ, McMahan CA, Seo E-J, Yu BP: The influence of dietary protein source on longevity and age-related disease processes of Fischer rats. J Gerontol Biol Sci 1988, 43:B5–B12
12. Lotzova E: Definition and functions of natural killer cells. Nat Immun 1993, 12:169–176
13. Cross RJ, Bryson JS, Roszman TL: Immunologic disparity in the hypopituitary dwarf mouse. J Immunol 1992, 148:1347–1352
14. Masoro EJ, Iwasaki K, Gleiser CA, McMahan CA, Seo E-J, Yu BP: Dietary modulation of the progression of nephropathy in aging rats: an evaluation of the importance of protein. Am J Clin Nutr 1989, 49:1217–1227
15. Cummings EA, Sochett EB, Dekker MG, Lawson ML, Daneman D: Contribution of growth hormone and IGF-1 to early diabetic nephropathy in type 1 diabetes. Diabetes 1998, 47:1341–1346
16. Doi T, Striker LJ, Gibson CC, Agodoa LY, Brinster RL, Striker GE: Glomerular lesions in mice transgenic for growth hormone and insulin-like growth factor-1. I. Relationship between increased glomerular size and mesangial sclerosis. Am J Pathol 1990, 137:541–552
17. Bartke A, Brown-Borg HM, Mattison JA, Kinney B, Hauck S, Wright C: Prolonged longevity of hypopituitary dwarf mice. Exp Gerontol 2001, 36:21–28
18. Corpus E, Harman SM, Blackman MR: Human growth hormone and human aging. Endocr Rev 1993, 14:20–39
19. Miller RA, Chrisp C, Atchley W: Differential longevity in mouse stocks selected for early life growth trajectory. J Gerontol A-Biol 2000, 55A: B455–B461
20. Khansari DN, Gustad T: Effects of long-term, low-dose growth hormone therapy of immune function and life expectancy of mice. Mech Ageing Dev 1991, 57:87–100