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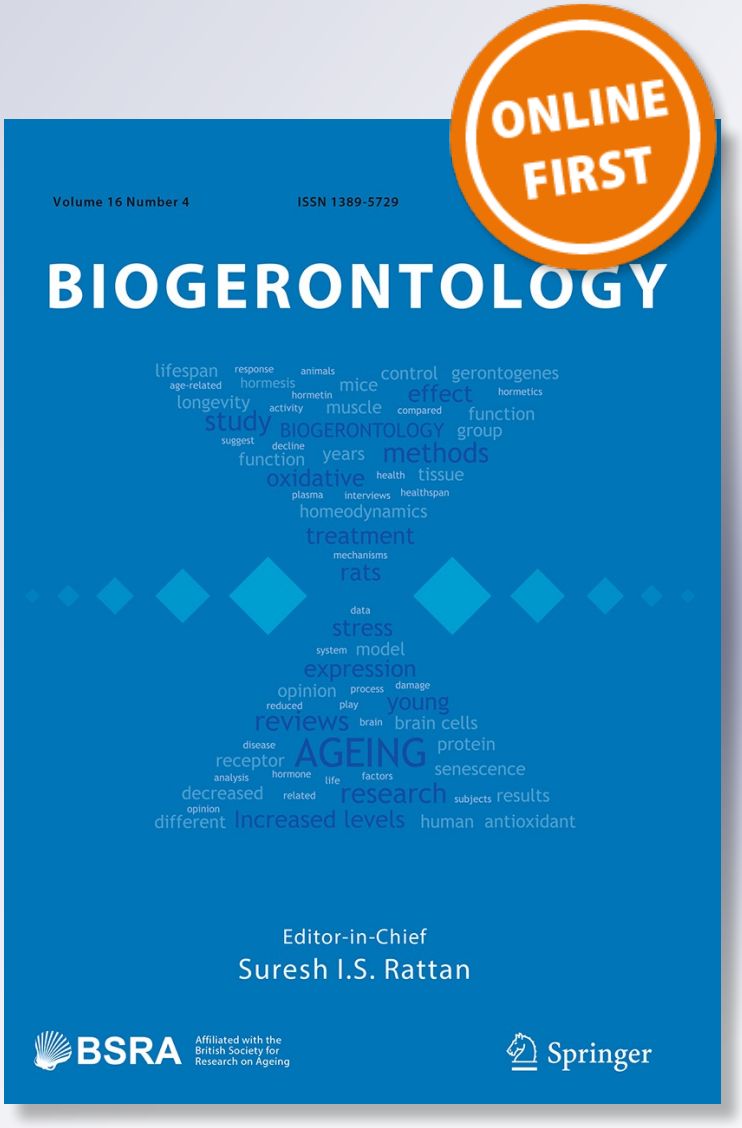


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REVIEW ARTICLE

# Trade off situation between thymus and growth hormone: age-related decline of growth hormone is a cause of thymic involution but favorable for elongation of lifespan

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**Abstract** High level of growth hormone (GH) is necessary for the activation of thymic function to promote T cell differentiation in the early stage of animal life. In the later stage of the life, administration of GH promotes the development of immune system and rejuvenates declined immune function of elderly people. By contraries, GH deficiency is favorable for the longer lifespan, as hypo-pituitary dwarf mice such as Ames and Snell dwarf mice exhibit longer lifespan than control. Furthermore over-expression of heterologous or homologous GH in transgenic mice shortens the lifespan. Ecuadorians carrying mutations of GH receptor gene are short in height, but exhibited low frequency of malignancy and no cases of diabetes. These data indicate that GH is necessary for the development of thymus dependent immune system but GH deficiency is favorable for long life span and decreases occurrence of cancer and DM. This situation is a kind of trade off situation between the immune system and GH. Thus the early decline of high level of GH occurring shortly after the birth is a cause of early decline of thymic functions, but favorable for longer lifespan. This situation could be a kind of trade off situation between thymus and GH.

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## Early decline of thymic function

Thymus plays an important role in the ontogenic development of T cell dependent immune system and it is well known that thymic involution starting at around puberty is one of major causes of the age-related decline of immune function. But precise examination in mouse models revealed that thymic function to promote T cell differentiation starts to decline in the early postnatal phase, earlier than the macroscopic involution (Hirokawa and Sado 1976).

The first information was obtained by assessment of anti-SRBC antibody response in TXB mice transplanted with thymus of syngeneic mice at various age (Hirokawa and Makinodan 1975). TXB mice was produced by thymectomy at 4 weeks of age and given 8.0 Gy of total body irradiation, followed by bone marrow transplantation. TXB mice were depleted in T cells and transplantation of thymus could recover T cells. Anti-SRBC antibody response was very low in TXB mice and increased in TXB mice transplanted with syngeneic thymus. The increase of anti-SRBC antibody was dependent on the number of helper T cells produced by transplanted thymus. In this experiment, TXB mice were transplanted with thymus

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obtained from newborn, 3 months old, 24 months old and 33 months old mice. Anti-SRBC antibody response showed age-related decline with age of thymus donor, indicating that the ability of thymus to promote helper T cells declined with advance of thymus age.

The experiment using nude mice with transplantation of aging thymus showed much more precise data (Hirokawa et al. 1982). The ability to promote killer T cells significantly decline at 2 weeks as compared with newborn thymus. In this series of experiment, it was shown that even old atrophic thymus had ability to promote $CD3^+$ T cell differentiation when grafted into nude mice. But when functionally assessed, these T cells were less active as compared with those produced by newborn thymus grafted into nude mice. When compared between newborn and 24 months old thymus, the magnitude of decline was tenfolds in helper function of anti-SRBC antibody response and 100 folds in killer T cell ability.

Emigration of thymic T cells to the spleen can be observed by intra-thymic injection of bone marrow cells into the thymus (Hirokawa et al. 1988). As this experiment was carried out using congenic mouse strain, B10.thy-1.1 and C57BL/6.Thy-1.2, T cells differentiated from injected bone marrow cells were clearly identified in thymus and spleen of the recipient mice. In this experiment, the number of donor type T cells in the spleen reflected both degree of proliferation and emigration of T cells differentiated from injected bone marrow cells within the thymus of recipient mice. The decline of donor-type splenic T cells was observed as early as 1 week of age and exponentially advanced thereafter; i.e., the emigration rate declined tenfolds at 6 months of age as compared with newborn stage.

These data showing the age-related decline of thymic function were obtained more than 30 years ago in mice. Since then, there are accumulating reports indicating that thymic involution is responsible for the age-related decline of immune functions (Palmer 2013; Dooley and Liston 2012; Taub et al. 2010; Lynch et al. 2009). In human cases, regeneration of $CD4^+$ T cells were examined in 15 patients (2 months–24 years of age) after intensive chemotherapy and the results showed that regeneration primarily occurred in children, but not enough in young adult (Mackall et al. 1995). Measurement of T cell receptor rearrangement excision circles (TRECs) could detect recent thymic emigrant population in human peripheral blood. The results showed that thymic output declined with age (Pido-Lopez et al. 2001). It is interesting to note that measurement of TRECs showed that thymic output was detected even in older individuals with an age-associated decline (Mitchell et al. 2010).

Thymectomy (TX) performed within 1 day after birth brings about immune deficiency as reported by Miller JFAP (1961). TX performed at day 3 after birth (day 3 TX) also causes immunological abnormality including autoimmune diseases. C3H/He mice were thymectomized 3 days after birth and then immunized at 4 weeks of age with a homogenate of the submandibular salivary gland emulsified in Freund's complete adjuvant (Hayashi et al. 1985). Inflammatory lesions developed in the salivary glands at 2 months and after. In autoimmune prone mice, such as NFS/sld, day 3 TX promoted the occurrence of inflammatory lesions in salivary and lacrimal glands (Haneji et al., 1994). Flow cytometric examination of spleen cells in NFS/sld mice revealed that $CD4^+Foxp3^+$ regulatory T cells quickly appeared within a week after birth and day 3 TX down-regulated the number of regulatory T cells promoting the occurrence of autoimmune diseases (Yamada et al. 2015).

Thymus peaks in size at around adolescence and starts to involute thereafter, suggesting that sex hormones negatively influence thymic size. Extirpation of testis and ovary (gonadectomy: Gx) was performed at 3, 6, 9, 15 and 18 months of age in male and female C57BL/6 mice (Utsuyama and Hirokawa 1989). Gx performed at any months of age increased the weight of thymus and the increase was more pronounced in male than in female mice.

Atrophy of thymus with immune deficient state was reported in hypo-pituitary Snell dwarf mice (Fabris et al. 1971) and Ames mice (Duquesnoy 1972). As pituitary gland is under the control of anterior portion of hypothalamus, the destruction of anterior portion of hypothalamus was expected to suppress pituitary function. The destruction of anterior portion of hypothalamus was performed in 3, 11 and 24 months old female Wistar rats. Contrary to the expectation, a significant increase of thymic weight was observed regardless of age, while a decrease was apparent in adrenal glands and ovaries (Utsuyama et al. 1997). It is interesting to note that serum level of growth hormone was greatly increased in the rats with destruction of anterior portion of hypothalamus; $182.3 \pm 7.0$ ng/ml

in experimental group and 9.4 ± 2.4 ng/ml in control group. Then, we examined the serum level of GH rats and mice from newborn to aged. The secretion of GH is known to occur in pulse-like fashion and the levels assessed were variable ranging from 2 to 15 ng/ml. While the level in newborn mice and rats were tenfolds higher than in those of other ages (Hirokawa et al. 1998). Growth hormone releasing hormone (GHRH) is a factor to promote the GH secretion and somatostatin (SST) is a factor to suppress GH secretion. We assessed quantitatively mRNA levels of GHRH and SST in hypothalamus of aging mice. The data indicated the GHRH decreased, while SST increased with advance of age (Hirokawa et al. 2001).

Thus, it is likely that the high level of GH is required for the growth and development of the thymus but maintained only in the short term after the birth and the level decreased thereafter.

## GH/IGF-1 and insulin/IGF-1 signaling

Requirement of GH for the development of immune system was first reported in congenitally hypopituitary mice; Snell (Fabris et al. 1971) and Ames (Duquesnoy 1972).

Administration of GH enhances thymopoiesis and therefore facilitate $CD4^+$ T cell recovery in HIV-1-infected adults (Chidgey 2008; Herasimtschuk et al. 2013). Treatment with GH induced thymic enlargement in a 7 years old girl with GH deficiency (Polgreen et al. 2007). In human adults, the somatotroph GH/IGF-1 axis is important for a normal thymus function (Morrhaye et al. 2009). On the one hand, it was found that Snell Dwarf mice lag behind their heterozygous littermates with respect to immune competence, but normal immune responsiveness develops in a delayed fashion (Cross et al. 1992). Furthermore, following survey reported that the lifespan of hypo-pituitary dwarf mice was longer than non-mutant control mice (Panici et al. 2010). They also showed that GH treatment of dwarf mice from 2 to 6 weeks of age caused significant somatic growth, but could not elongate their lifespan.

Findings in transgenic mice of GH and GHRH are striking (Bartke et al. 1999: Dialynas et al. 1999). As expected, the overexpression of heterologous and homologous GH can lead to giant body with hyperplasia of thymus and spleen, and increased mitogenic response of spleen cells. But the giant mice exhibit a short lifespan and impaired glucose metabolism (Benencia et al. 2014).

The reduced lifespan in GH transgenic mice is multifactorial but consistent with the elongation of lifespan in hypopituitary dwarf mice, mentioned above.

Growth hormone binds GH receptor on the cell membrane and induces the secretion of insulin-like growth factor-1 (IGF-1). IGF-1 binds IGF-1 receptor on the cell membrane which has similar cell activation signal with insulin receptor. Lifespan of animals (*C. elegans*, *Drosophila* and mice) can be elongated by mutations that diminish insulin-IGF-1 signal pathways (van Heemst 2010). This is consistent with the fact that the decrease of GH–IGF-1 signal in Snell and Ames dwarf mice has longer lifespan. Mutant animals characterized by extended longevity showed improved insulin signaling and carbohydrate metabolism, and decreased pro-inflammatory activity (Masternak and Bartke 2012).

An interesting report in human was the monitoring of 90 Ecuadorian subjects who carry mutations in the growth hormone receptor gene that lead to severe GHR and IGF-1 deficiencies. They exhibited only one non-lethal malignancy and no cases of diabetes (Guevara-Aguirre et al. 2011).

A study of female centenarians in Ashkenazi Jewish cohort suggested that altered IGF signaling pathway play a role in modulation of human lifespan (Suh et al. 2008). The results showed that overrepresentation of heterozygous mutations in the genes in the IGF1R gene among centenarians relative to controls that are associated with high serum level of IGF1 and reduced activity of the IGF1R as measured in transformed lymphocytes.

## Conclusion

The data described above have indicated that high level of GH is essential for the development of thymus dependent immune system. The high level of GH decreases shortly after birth to adult level which is required for the growth of bone and muscle. While at the same time, many data in various animals have indicated that GH–IGF signaling is a factor reducing life span. Even in human, the GH–IGF signal pathway is closely related with occurrence of various diseases.

In fact, diabetes mellitus and cancer are rare in a dwarf population lacking GH-R. In a simple term, GH is required for the development and maintenance of the immune system, but promote occurrence of some diseases by different pathway which is not directly related with the immune system. Thus, we can see here a kind of trade-off situation between thymus and growth hormone.

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