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Expression and function of chicken bursal growth hormone (GH)

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ABSTRACT

Growth hormone (GH) has several effects on the immune system. Our group has shown that GH is produced in the chicken bursa of Fabricius (BF) where it may act as an autocrine/paracrine modulator that participates in B-cell differentiation and maturation. The time course of GH mRNA and protein expression in the BF suggests that GH may be involved in development and involution of the BF, since GH is known to be present mainly in B lymphocytes and epithelial cells. In addition, as GH is anti-apoptotic in other tissues, we assessed the possibility that GH promotes cell survival in the BF. This work focused on determining the mechanism by which GH can inhibit apoptosis of B cells and if the PI3K/Akt pathway is activated. Bursal cell cultures were treated with a range of GH concentrations (0.1–100 nM). The addition of 10 nM GH significantly increased viability (16.7 ± 0.6%) compared with the control and decreased caspase-3 activity to 40.6 ± 6.5% of the control. Together, these data indicate that GH is produced locally in the BF and that the presence of exogenous GH in B cell cultures has antiapoptotic effects and increases B cell survival, probably through the PI3k/Akt pathway.

1. Introduction

It has been proposed that various peptide messengers, such as hormones and growth factors, can be expressed ectopically and may act as paracrine and/or autocrine factors and exert local regulatory effects on cellular communication.

While growth hormone (GH) is primarily produced in the pituitary gland, it is well established that the GH gene is also expressed in many other tissues, including those of the immune system (Clark, 1997; Gelato, 1993; Hattori et al., 1999; Johnson et al., 1997). Previous studies have shown that GH, through circulating and locally produced insulin growth factor type 1 (IGF-1), plays a direct and indirect regulatory role in the development and function of the immune system including: immunoglobulin secretion by B cells, thymulin secretion of thymic epithelial cells, NK cell activity, phagocytosis, and the killing capacity of neutrophils and macrophages (Clark, 1997; Kooijman et al., 1997; Postel-Vinay et al., 1997; Sumita et al., 2005; Weigent et al., 1988). Moreover, GH deficiency impairs immune function by reducing thymic size and bursal development as well as cellular and humoral immune responses (Kelly et al., 2007; Dorshkind and Horseman, 2000; Johnson et al., 1993), which can be restored by addition of exogenous GH (Johnson et al., 1997; Khansari and Gustad, 1991). GH can also stimulate growth in primary (thymus and bursa of Fabricius) and secondary (spleen) immune organs (Marsh, 1992; Villanua et al., 1992), induce lymphocyte proliferation, and increase the production of cytokines and other immune factors (Johnson et al., 1997; Murphy et al., 1993; Yoshida et al., 1992).

2. GH effects on the immune system

In a variety of organisms, GH deficiency due to genetic (dwarfism), physiological (aging), or experimental (hypophysectomy) causes has been associated with reduced immune function; rodents and humans show atrophy of the thymus and the peripheral lymphoid organs, a decreased number of splenic T and B cells, and depletion of cells in the bone marrow (Dorshkind and Horseman, 2000; Kincade et al., 1970; Kooijman et al., 1995; Murphy et al., 1993; Weigent and Blalock, 1995), which can be corrected by GH. When exogenous GH is administered in dwarf mouse, it increases B and T lymphocytes (CD4+, CD8+) and macrophage activation, and in humans it increases the production of immunoglobulin and the activation of natural killer cells and macrophages (Clark, 1997; Hattori et al., 1999; Johnson et al., 1997; Kelly et al., 2007; Kooijman et al., 1996, 1995). On the other hand, pathological increases in the concentration of GH (e.g., acromegaly due to pituitary adenomas) stimulate the activity of the immune system and also induce hyperplasia of the thymus and spleen (Harvey and Hull, 1997; Jeay et al., 2002). It is now well established that GH and IGF-I play an important role in maintaining the integral

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function of the immune system and are involved in mechanisms of proliferation and differentiation of mammalian immune cells, such as lymphocytes, macrophages, and thymocytes (Weigent et al., 1988; Weigent and Blalock, 1991, 1995); in birds, these cells may be similarly modulated directly by GH or indirectly by IGF-I (Render et al., 1995). Moreover, hypophysectomy in birds induces atrophy of the thymus and bursa of Fabricius (BF), but when GH is replaced, renewed growth is observed in these tissues (Johnson et al., 1993; Marsh, 1992). However, these experiments found that the response of the thymus occurs in the absence of changes in the bursa, while the bursal response occurs in the absence of changes in the thymus, which indicates that these differential responses can be due to tissue-specific differences, or to different doses or temporal sensitivity to GH (Hull and Nette, 2001).

Lack of GH affects the differentiation, proliferation, and function of certain lymphoid cells, such as thymocytes and lymphocytes, which are reduced in number within 3 weeks after hypophysectomy in the domestic fowl (Johnson et al., 1993). GH-resistant dwarf chickens show some deficiencies in immune function such as low levels of thymulin and a reduced proliferation response by T lymphocytes (Kooijman et al., 1995). It has been suggested that certain T-cell subpopulations of the thymus respond to GH at specific stages of development by increasing the proportion of CD4+ and CD8+ thymocytes at the expense of immature CD1 cells in cases of autoimmune thyroiditis in chickens (Marsh et al., 1992). Similarly, peripheral blood lymphocytes treated with GH are more sensitive to the proliferation induced by Concanavalin A or Phytohaemagglutinin (PHA), which affect different populations of T cells (Marsh, 1992). The administration of exogenous GH improves the immune humoral response in GH-resistant dwarf chickens, but the mechanism is not yet clear (Harvey and Hull, 1997; Hull and Nette, 2001; Johnson et al., 1993; Marsh, 1992).

The immune-modulatory effect of GH may be mediated in part by cytokines (Chandratilleke et al., 1994) or it may reflect an endocrine action of pituitary GH, transported by bloodstream, on GH receptors expressed in lymphoid tissues. However, recent reports that GH is synthesized in chicken primary (thymus and bursa of Fabricius) and secondary (spleen) immune organs suggest the possibility that locally produced GH can also act in an autocrine or paracrine manner to regulate immune function (Hull and Nette, 2001; Luna et al., 2005, 2008). Such actions would not be mediated by IGF-I, at least not in the spleen, since IGF-I transcript abundance in the spleen of birds is not affected by GH treatment. Moreover, the GH-resistant dwarf chickens are not deficient in IGF-I mRNA produced by the spleen (Brooks et al., 2008; Conway-Campbell et al., 2007; Costoya et al., 1999; Hull and Nette, 2001; Lanning and Carter-Su, 2006). The nuclear localization of an immunoreactive complex similar to the GH–GH receptor (GH-GHR-IR) may also indicate GH intracrine action, in which immune GH acts on nuclear receptors without leaving the secretory cell (Brown, 2005; Conway-Campbell et al., 2007; Jeay et al., 2002).

Previous reports support the proposal that GH acts as a cytokine in the immune system under stress conditions, since it counteracts the immunosuppressive effect of glucocorticoids in T lymphocytes (Jeay et al., 2002). GH can stimulate the cell cycle of lymphoid cells (proB Ba/F3 cell line transfected with the GH receptor, T cells), HL-60 cells, and cardiomyocytes, and it can prevent apoptosis, which was shown to be mediated via phosphatidylinositol 3′(PI3K) kinase/Akt (a serine/threonine kinase), the transcription factor NF-kB, and the expression of the anti-apoptotic protein Bcl-2 (Jeay et al., 2002, 2001). GH is able to regulate the expression of several mediators of the cell cycle such as, c-Myc and cyclins A and E (Jeay et al., 2001). These findings open up new alternatives for the mechanisms of GH action in controlling apoptosis, proliferation, differentiation, and development of cells in the immune system, but aberrant expression of this hormone in immune cells has been found to promote transformation, suggesting that the over-expression of GH may lead to development of immune neoplasias (lymphomas, melanomas, and T-cell leukemias) (Costoya et al., 1999; Jeay et al., 2002, 2000; Kooijman et al., 1996; Rayet and Gelinas, 1999).

3. Expression of GH in the immune system

In recent years, evidence has accumulated showing that GH can be expressed in the immune system. The mRNA coding for this protein was shown to be identical to that expressed in the pituitary gland. This has been described in comparison with GH mRNA in rat lymphocytes (Rohn and Weigent, 1995); in human leukocytes (Hattori et al., 1999; Liu et al., 1997; Melen et al., 1997; Wu et al., 1999); in bone marrow, spleen, and thymus of rodents (Weigent et al., 1988; Weigent and Blalock, 1991, 1995); in dogs (Lantinga van Leeuwen et al., 2000); in cattle (Chen et al., 1997); and in chicken lymphoid tissues (thymus, spleen, and bursa of Fabricius [BF]) (Luna et al., 2005, 2008; Render et al., 1995; Rodríguez-Méndez et al., 2010). Both GH mRNA and a protein similar to GH were expressed in lymphoid organs and peripheral blood lymphocytes, particularly in B cells of 4-week-old chickens (Render et al., 1995).

The concentration of GH in the BF is much lower (<0.5%) than that found inside the pituitary gland; however, important changes in GH concentration were observed in the BF during chicken development (Luna et al., 2005). The bursa of Fabricius undergoes striking changes in size during development, growing rapidly during late embryogenesis and for several weeks after hatching, before regressing in sexually mature adults (Ciriaco et al., 2003; Glick, 1991). Content of pituitary GH in 4-week-old chicken is 28.9 μg GH/organ, and inside the bursa of Fabricius the content, even this tissue is larger than the pituitary, was about 22 μg GH/organ (Hull et al., 2005). The changes in expression and content of both GH mRNA and protein in the BF were found to parallel the developmental growth pattern of this organ, increasing steadily from embryonic day 15 (ED 15) until the juvenile stage (6–8 weeks old), the phase of most rapid growth of the BF, and then decreasing significantly during the atresia phase (18–20 weeks old) when reaching sexual maturity, suggesting that GH may be involved in BF development and involution (Fig. 1) (Luna et al., 2005, 2008; Rodríguez-Méndez et al., 2010). GH-IR was mainly found in IgM-expressing cells during the embryonic stage, while it was highest in IgG-expressing cells after hatching (Rodríguez-Méndez et al., 2010). These data suggest that GH may also play a role during maturation of B-cells, which are known to undergo a switch from IgM to IgG expression during early development in the chicken (Cooper et al., 1972; Hull and Nette, 2001; Kincade and Cooper, 1971; Kincade et al., 1970).

These age-related changes have also been described in the pituitary where, besides the monomer (26 kDa under reduction conditions), other molecular variants between 15–52 kDa were found, are thus likely to include translational variants (e.g. glycosylated and phosphorylated forms), proteolytic cleavage fragments (e.g. the 15 kDa variant), protein bound forms and oligomers (including dimer and trimer forms) and also have shown expression patterns that change with age (Arámburo et al., 2001). In the BF, we have detected GH molecular variants (14, 17, 26, 29, 32, 37, 40, 46, 52 kDa) whose relative proportions change throughout ontogeny in male chickens; initially the GH moiety with a molecular weight of 17 kDa was the most abundant (Luna et al., 2005, 2008), and it is of interest that seven of these variants are present in pituitary except the 17 kDa variant. However, this isoform (17 kDa) is the most abundant GH variant in several extrapituitary tissues like chicken testis and ovarian follicles (Ahumada-Solórzano et al., 2012) This suggests that GH transcript translation or processing of the mature hormone to sub-monomeric form (e.g., by proteolytic cleavage),