Transgenic mice expressing bovine GH develop arthritic disorder and self-antibodies

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Abstract

We observed disability of movement in 6-month-old transgenic mice expressing the fusion gene coding for the bovine GH (bGH) under the transcriptional control of phosphoenolpyruvate carboxykinase promoter (PEPCK-bGH). Histological study of the knee joint showed altered synovial and tibial articular cartilage tissues. In the cartilage the following observations were made: (i) generalized loss of the normal zonal structure and presence of clefts, and (ii) profound alterations in chondrocyte growth/differentiation processes consistent with hypertrophy. The synovial tissue showed a reduced number of adipocytes, and a significant thickening of synovial lining tissue and pannus. These findings indicate that transgenic mice suffer damage to diarthritic joints with osteoarthritic appearance.

As changes in synovial membrane in osteoarthritis are almost indistinguishable from those seen in inflammatory arthritis, we determined the potential correlation with an immunological disorder. Serological determination of self-antibodies measured as a function of age and sex showed anti-nuclear, anti-single-stranded DNA, anti-double-stranded DNA and anti-70K antibodies, and an altered immunoglobulin typing. These results suggest that transgenic mice expressing bGH develop an arthritic process which is correlated with an immune disorder. The results also indicate that these mice are a suitable animal model to study the specific role of GH-driven processes in immune cells and arthritis.

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Introduction

Growth hormone (GH) has long been recognized as being essential for somatic growth, differentiation, metabolism and thymic development (Clark 1997, Barkan 1992). The GH receptor (GHR) is a member of the cytokine/haematopoietin family of receptors (Kelly *et al.* 1993). The availability of hormone-deficient mice has renewed the interest in the effect of hormones on functional lymphocytic response. Studies performed in the Snell dwarf (dw/dw) strain of mice, deficient in GH, thyroid-stimulating hormone (TSH), prolactin (PRL) and indirectly in insulin-like growth factor-I (IGF-I), revealed that their thymus was atrophied and their bone marrow was markedly hypocellular (Li *et al.* 1990, Murphy *et al.* 1992a). Later on, cytometric analysis showed that the production of CD4⁺CD8⁺ thymocytes ceased at early ages in these mice, and that bone marrow B cell population showed depressed progenitor pool levels of CD45R⁺/surface IgM⁺ (Murphy *et al.* 1992b, Montecino-Rodríguez *et al.* 1996). This helps to explain the abnormally low numbers of marrow mononuclear cells in dwarf mice.

Studies carried out in IGF-I^{-/-} animals (Lin *et al.* 1993) and (lit/lit) mice that have a defect in the gene encoding the receptor for hypothalamic GH-releasing factor and consequently present low GH/IGF-I levels (Powell-Baxton *et al.* 1993), support the notion of a general anabolic role of GH and IGF-I in thymus and bone marrow. When these mice were treated with IGF-I and GH respectively, the absolute number of marrow myeloid and B and thymic T cells increased. However, the relative increase was not significant taking into account the overall weight gain of the animals treated with GH and IGF-I (Montecino-Rodríguez *et al.* 1997). On the other hand, induction of lymphopoiesis and haematopoiesis *in vivo* and *in vitro* by GH and IGF-I has been demonstrated in a number of works (Clark 1997) and the expression of GHR and IGF-I receptor in immune cell system has been reported in human and murine species (Kooijman *et al.* 1995, Gagnerault *et al.* 1996, Mello-Coelho *et al.* 1998).

Transgenic (Tg) mice expressing GH from different species under different promoters have been generated (Frohman 1996). They present high systemic GH levels and in addition to increased skeletal growth, several other

organs and systems are direct or indirect targets. They present renal pathology, diabetes, hypertension, sterility and several neuropathies (Quaife *et al.* 1989, Frohman 1996). However, the precise pathogenesis involved or whether the immune system is affected is still not known. The phenotypic characterization of lymphoid organs in 2-month-old phosphoenolpyruvate carboxykinase promoter (PEPCK-bGH) Tg mice has been reported (Gonzalo *et al.* 1996). Significant alterations in T-cell functions were observed, suggesting a potential role for GH in regulating the immune response. In addition, while T-cell subpopulations were found with minor changes, bone marrow was deprived of pre-B cells (Gonzalo *et al.* 1996).

In bGH Tg mice older than 6 months we observed disability of movement suggestive of the development of an arthritic process. Kidney damage, arthritic processes and the presence of self-antibodies are manifestations characteristic of an autoimmune disorder (Theofilopoulos & Dixon 1985). Since bGH Tg mice develop glomerulosclerosis (Quaife *et al.* 1989), we considered it of interest to characterize the type of lesion of the knee joint and the status of the immune system. Histological determination of the knee showed profound alterations of the articular cartilage and synovial tissue resembling an arthritic process. Results of serologic determinations were consistent with the spontaneous development of an immunological disorder. The possible relationship between high GH levels and autoimmunity and the possibility that the articular alterations corresponded to an osteoarthritic or rheumatoid process are discussed. Our results suggest that bGH Tg mice are a suitable animal model to address the pathogenesis of arthritic processes and the mechanism by which sustained high GH/IGF-I levels cause immunological disorders. In addition, the side-effects described in humans following exogenous GH administration such as oedema and a subclinical activation of lupus nephritis (Yap *et al.* 1998) are related to the bGH Tg murine model.

Material and Methods

Animals

Transgenic mice were generated by M M McGrane (McGrane *et al.* 1990). The bGH Tg line was maintained by mating male transgenic animals with C57BL6 females for 15–18 generations (Gonzalo *et al.* 1996). Mice were reared under the classical barrier system in the animal house of the Centro de Biología Molecular. Transgenic mice showing increased body size were referred to as bGH Tg mice and were compared with litter mates of normal size referred to as control mice.

Histologic studies

Proximal tibia epiphyses were placed in 10% formalin, decalcified at room temperature in a 0.07% EDTA pH 7.2

solution for 24–48 h and processed for paraffin embedding. A series of 5 µm-thick sections were stained with haematoxylin-eosin (HE) following standard procedures. Sections were evaluated for the presence of inflammatory infiltrates, synovial proliferation and hyperplasia with or without exudates, fibrinoid inflammatory nodules, vasculitis, arteritis in periarticular and articular regions, and for chondrocyte differentiation stages (Mankin *et al.* 1994). Photographs were taken with an Oxford Trade photomicroscope, using Kodak TMAX 100 film.

Immunoprecipitation and immunoblotting

A 100 µl volume of serum was diluted fourfold with PBS, adjusted to 1% Triton X-100 and incubated at 4 °C with 5 µg of rabbit anti-mouse (RAM) (Nordic Immunology Laboratories, Tilburg, The Netherlands) bound to Protein A-Sepharose (Sigma, St Louis, MO, USA) for 1 h. Immune complexes were washed as follows: three times with buffer A (10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 50 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulphonylfluoride, 2 mg/ml leupeptine and 0.5 mg/ml benzamidine), once with 0.5 M LiCl, and twice with a buffer containing 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 100 mM NaCl. The clean immune complexes were dissociated by addition of a twice-concentrated SDS-gel loading buffer and by boiling for 10 min. Samples were analysed by SDS-PAGE. Gels were transferred to nitrocellulose (Schleicher and Schuell, Dassel, Germany). The nitrocellulose membranes were blocked at 37 °C for 2 h with Tris-buffered saline (TBS) containing 5% milk proteins and 0.05% Tween 20. The blotted proteins were probed for 70K by incubating overnight at 4 °C with MAb H111 (Kastner *et al.* 1992) (generously provided by Dr R Lührmann, Malburg, Germany) diluted 1:100 in TBS containing 5% non-fat BSA or milk proteins. The second antibody used for detection was an anti-mouse labelled with horseradish peroxidase. Blots were washed and developed using the enhanced chemiluminescence (ECL) system (Amersham Iberica, Madrid, Spain) according to manufacturer's instructions. Over 80 blood serum samples from bGH Tg mice were analysed. The percentage of animals presenting anti-70K antibodies was analysed on the basis of age and sex.

Serological studies

Anti-single-stranded DNA (ssDNA) and anti-double-stranded DNA (dsDNA) were measured by the enzyme-linked immunosorbent assay (ELISA) method. Briefly, 96-well plates were doubly coated with 50 µl poly-L-lysine at 25 mg/ml in PBS and subsequently with 50 µl salmon sperm DNA or mouse DNA at 20 mg/ml. Each coating step was carried out incubating at 37 °C for 1 h. Wells were then blocked with 300 µl of 5% BSA in PBS at 37 °C for 2 h. Blocked wells were reacted with 50 µl of 1/1000

Figure 1 Cartilage examination. Articular cartilage of control mice (A) and bGH Tg mice (B, C, D). Cartilage of bGH Tg mice has frequent clefts (B). Transgenic mice show disorganized deep chondrocytes in the radial zone (r of A versus C and D). Chondrocytes at the transitional zone show hyperthropy in bGH Tg mice (t in A versus C and D). Elongated chondrocytes at the superficial zone (s in A disappear in bGH Tg mice (C and D). oz, osification zone; sb, subchondral bone. $\times 1000$ magnification.

serum at room temperature for 2 h. Between incubations, wells were thoroughly washed with PBS-0.1% Triton X-100. The bound antibody was quantified using an anti-mouse antibody labelled with horseradish peroxidase. Peroxidase activity was measured using O-phenylenediamine (OPD) following the manufacturer's instructions (Sigma, St Louis, MO, USA). Mouse immunoglobulin isotypes were determined by ELISA using a Southern Biotechnology (Birmingham, AL, USA) kit. Goat anti-mouse (L+H) antibody at 10 mg/ml was used as a coating reagent. Quantification was carried out using 50 μ l of aliquots 1/10 to 1/4000 serially diluted of each standard antibody isotype. Serum samples were diluted 1/25 000 in PBS.

Statistical analysis

Densitometric intensities of sera from bGH Tg mice were expressed in units of standard deviation from the mean intensity of the group of control sera. A single value of mean intensity per serum was calculated by averaging the

corrected intensities in all determinations. A serum was considered to be immunoreactive when its mean intensity was higher than 95% ($P < 0.05$) of the mean intensity of the control sera. Statistical analyses of frequencies were performed by the χ^2 test. Logistic regression analysis, relative incidences and 95% confidence intervals (CI) were calculated as previously described (Morris & Gardner 1988).

Results

Histological study of the knee of bGH Tg mice

At 6 months old, bGH Tg mice showed impaired movement. We assessed the histological characterization of the proximal tibia region of 40 male and female animals over 6 months and compared results with those obtained from control animals. As expected, the chondroepiphysis of bGH Tg mice was twice the size of the control mice. In Fig. 1A, the three characteristic zones of a normal articular cartilage were observed in control animals: the radial zone

(r), which involves a small number of primary cartilage cells; the transitional zone (t), composed of primary cells at the stage of proliferation and acquiring the chondrocyte phenotype; and the superficial zone (s), which is composed of two lines of elongate chondrocytes and constitutes the upper gliding surface of joints. In contrast to control mice, articular cartilage was altered in all the bGH Tg mice examined. Discontinuous regions of cartilage composition were observed along the tibial joint: regions presenting a significant reduction in thickness (Fig. 1C), regions where articular cartilage was absent (data not shown), regions with a regular thickness (Fig. 1D) and regions with clefts (Fig. 1B). The chondrocyte pattern of articular cartilage was altered in bGH Tg mice. As can be observed in Fig. 1C and D, in the deep radial zone (r) chondrocytes were usually disorganized in comparison with control cartilage. Significant changes were also detected in the transitional zone (t): the number of chondrocytes at the end of clonal expansion (two nuclei), aligned chondrocytes and single differentiated chondrocytes were significantly reduced and the number of hypertrophic chondrocytes increased (Fig. 1C), while regions of articular cartilage with a normal thickness (Fig. 1D) presented an increased number of chondrocytes at all stages of clonal expansion and hypertrophied chondrocytes. Interestingly, the superficial zone(s) of articular cartilage disappeared in bGH Tg mice (Fig. 1C and D). This zone is composed of highly hypertrophied chondrocytes with large dense nuclei instead of the two lines of elongate chondrocytes present in control mice.

The synovia of bGH Tg mice (Fig. 2B and C) also presented profound alterations in comparison with control animals (Fig. 2A). A reduced number of adipocytes (a) and a thickening of the synovial lining layer (lt) was generally observed in comparison with control synovial tissue (Fig. 2, A versus B). Vasculitis and/or arteritis and inflammatory infiltrations were occasionally observed although pannus was often present (Fig. 2C).

Presence of anti-nuclear antibodies in bGH Tg mice

The effect of chronic circulating GH on the immune system was assessed by determining in bGH Tg mice the main serological parameters described in murine models of autoimmune diseases (Theofilopoulos & Dixon 1985). Four- to six-month-old Tg-positive mice of both sexes were studied for the presence of self-antibodies. A total of 24 sera from Tg mice (both sexes) and 24 sera from control mice were probed on NIH 3T3 cell preparations. More than 90% (22/24) of the tested sera from Tg-positive mice reacted to the nucleus of NIH 3T3. Conversely, only 1 of the 24 control sera reacted to the NIH 3T3 nucleus. These results prompted us to study the incidence of anti-ss and anti-dsDNA antibodies in bGH Tg mice compared with control mice. A systematic study of immunoreactivity to ssDNA and dsDNA was then performed, using sera from 30 control and 60 bGH Tg mice. Densitometric data were

Figure 2 Synovial examination. Synovial tissue of bGH Tg mice (B) shows a reduced number of adipocytes (a) and thickening of synovial lining tissue (lt) with uncovered zones of cells (*) when compared with control mice (A). Pannus observed in bGH Tg mice (C). $\times 1000$ magnification.

quantified and processed as described in the Material and Methods section. The results obtained are summarized in Fig. 3. We found immunoreactivity to ssDNA in 33% ($P < 0.02$) and to dsDNA in 25% ($P < 0.05$) of bGH Tg. Sera of these mice yielded a staining intensity above the 95% confidence level compared with control group.

We also studied whether anti-ssDNA and dsDNA antibody levels were affected by age considering three age groups (2 months (Y), 4–6 months (A) and 6–8 months (O)) of bGH Tg animals. The frequency of immunoreactive sera to ssDNA at the 95% confidence level was

Figure 3 Incidence of anti-ssDNA and anti-dsDNA antibodies. Mean immunoreactivity to ssDNA and dsDNA of control (○) and bGH Tg sera (●). Circles represent the mean immunoreactivity of each serum in terms of densitometric intensity. Horizontal, broken lines indicate the 95% superior confidence limits of the mean. In A, sera of control (C) and bGH Tg mice are shown. In B, sera of bGH Tg mice at different ages are shown: Young (Y), 2 months; Adult (A), 4–6 months; Old (O), 6–8 months. In C, sera of bGH Tg mice, both males (M) and females (F), are shown.

found to be significantly higher in both adult (A) and old (O) Tg mice groups in comparison to that of the young (Y) Tg mice group ($P < 0.04$) and there was no significant difference between frequencies in the adult and old Tg mice groups ($P > 0.05$). On the other hand, the frequency of immunoreactive sera to dsDNA was found to gradually increase with age considering the above-mentioned three groups of bGH Tg mice and was, therefore, analysed using a logistic regression model. This analysis yielded a significant 2.8-fold (1.4–5.8, 95% CI) and 8.0-fold (1.9–33.6, 95% CI) increase in the incidence of immunoreactive sera of the adult and old groups of Tg mice considering the group of young mice as a reference group. The correlation between sex and immunoreactivity to ssDNA and dsDNA was found to be higher in females than males ($P < 0.005$ and $P < 0.05$ respectively) (Fig. 3).

Anti-70K antibodies in bGH Tg mice

The above results suggested the development of an autoimmune disorder. To further analyse this possibility, we tested for the presence of other autoimmune markers such

Figure 4 Incidence of anti-70K component of U1 antibodies. 100 µl of serum samples from bGH Tg mice (72 animals) and control mice (68 animals) of both sexes were reacted with proteins isolated from mouse liver nuclei. Immune complexes were analysed by Western blotting and probed for 70K protein using MAb H111. Detection was carried out chemiluminescently. The upper part of the figure shows the results of a representative determination where the 70K is indicated (A). The lower part of the figure shows the results expressed as percentages (B). Anti-70K antibodies in sera of bGH Tg mice in adult, 4–6-month-, and old, 6–8-month-old groups of males (M) and females (F) are shown. Anti-70K antibodies in sera of control (C) mice were only observed in females (4/68) of the 6–8-month-old group.

as anti-70K (Lerner & Stein 1979). The 70K protein is a component of U1 small nuclear ribonucleoprotein (U1snRNP) which plays an essential role in pre-mRNA splicing (Kastner *et al.* 1992). To this end, sera were used to precipitate proteins extracted from isolated mouse liver nuclei and the presence of the 70K protein in immune complexes detected using MAb 111 by Western blotting. In Fig. 4A a determination of 70K is shown as an example.

Table 1 Immunoglobulin typing. The levels of IgM and IgG subclasses in young and old bGH Tg mice and control mice of both sexes (12 animals of each category) were determined by ELISA using a kit from Southern Biotechnology. Quantifications were carried out using the corresponding standard Ig. Data are means $\pm$ S.E.M. Underlined values: $P < 0.05$ versus control values

Serum Immunoglobulin Levels (mg/ml)								
	Young				Old			
	Control		bGH Tg		Control		bGH Tg	
	M	F	M	F	M	F	M	F
IgM	0.11 $\pm$ 0.02	0.08 $\pm$ 0.01	0.17 $\pm$ 0.02	<u>0.29 $\pm$ 0.03</u>	0.47 $\pm$ 0.02	0.30 $\pm$ 0.00	0.58 $\pm$ 0.12	0.44 $\pm$ 0.08
IgG	0.81 $\pm$ 0.04	0.90 $\pm$ 0.02	1.04 $\pm$ 0.17	<u>1.36 $\pm$ 0.18</u>	1.95 $\pm$ 0.04	1.66 $\pm$ 0.05	1.76 $\pm$ 0.18	1.88 $\pm$ 0.19
IgG ₁	0.40 $\pm$ 0.03	0.48 $\pm$ 0.02	0.43 $\pm$ 0.12	0.63 $\pm$ 0.05	0.69 $\pm$ 0.02	0.41 $\pm$ 0.01	0.56 $\pm$ 0.20	0.65 $\pm$ 0.13
IgG _{2a}	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	<u>0.06 $\pm$ 0.01</u>	<u>0.08 $\pm$ 0.03</u>	0.32 $\pm$ 0.01	0.37 $\pm$ 0.02	0.32 $\pm$ 0.03	0.24 $\pm$ 0.12
IgG _{2b}	0.30 $\pm$ 0.02	0.33 $\pm$ 0.01	0.40 $\pm$ 0.14	0.48 $\pm$ 0.02	0.66 $\pm$ 0.01	0.60 $\pm$ 0.02	0.59 $\pm$ 0.04	0.61 $\pm$ 0.04
IgG ₃	0.10 $\pm$ 0.01	0.08 $\pm$ 0.01	0.15 $\pm$ 0.01	<u>0.17 $\pm$ 0.01</u>	0.29 $\pm$ 0.01	0.28 $\pm$ 0.01	0.29 $\pm$ 0.01	0.39 $\pm$ 0.08

We observed that, at early ages (2 months and both sexes) the presence of anti-70K antibodies in sera of bGH Tg mice was not significant (results not shown). However, the percentage of bGH Tg mice displaying anti-70K antibodies in the 4–6- and 6–8-month-old groups of males and females was surprisingly high (Fig. 4B). The incidence of anti-70K antibodies was 85–90%, irrespective of age and sex. In control mice, only the 6–8-month-old group of animals showed a sporadic presence of anti-70K antibodies (4/68).

Increased levels of IgM, IgG_{2a} and IgG₃

The level of circulating Ig subclasses in young (2–3 months old) (24 animals) and old (8–9 months old) (24 animals) male and female bGH Tg mice compared with controls was also studied. As shown in Table 1, young Tg males presented 1.5-, 6- and 1.5-fold increases in IgM, IgG_{2a} and IgG₃ respectively, while these increases were approximately double in Tg females (3.6-, 8- and 3.6-fold, respectively). The total IgG concentration in young bGH Tg mice increased by 23% in males and 40% in females. Nevertheless, these differences were not evident in bGH Tg old mice since control mice already displayed high levels of immunoglobulins (Table 1).

Discussion

The results of this work show that bGH Tg mice develop arthritic joints. Both articular cartilage and synovial tissues showed severe damage including a generalized loss of the normal zonal structure of cartilage and frequent cartilage clefts, altered chondrocyte growth/differentiation pattern, a decrease in synovial adipocytes and proliferation of synovial lining cells. Moreover, we show that bGH Tg mice develop self-antibodies in serum including anti-70K, anti-ssDNA, anti-dsDNA and altered immunoglobulin typing as a function of age and female sex that suggest an

autoimmune-like disease. Articular and immune disease can be interpreted as independent phenomena as a consequence of a direct effect of sustained high GH/IGF-I levels, or articular damage dependent on the development of an autoimmune-like disease.

The role of GH/IGF-I in joints can be explained by increasing evidence that the integrity of joints is maintained by the balance of cytokine-driven processes in bone, cartilage and synovium. Unregulated or excess influences of these molecules are thought to play a part in the pathophysiology of joint diseases (Westcott & Sharif 1996). However, the synovia appears to play a predominant role in diarthrosis. A thickening of synovial lining tissue and an altered rate of some cytokine production by these cells has been shown in both patients with osteoarthritis and rheumatoid arthritis (Gay *et al.* 1993, Smith *et al.* 1997). bGH Tg mice show proliferation of synovial lining cells similar to that observed in the synovial membranes from patients with severe osteoarthritis and may produce an altered rate of cytokine in the knee joint. On the other hand, synovial hyperplasia, inflammation and autoimmune phenomena are well known hallmarks in rheumatoid arthritis (Gay *et al.* 1993). Since inflammatory infiltrates are rarely seen in synovial tissue of bGH Tg mice, this suggests that the alterations observed could be related to an osteoarthritis process. However, a rheumatoid arthritis process can not be excluded given that a T-cell independent pathway has been demonstrated where synovial lining cells attach, invade and destroy cartilage by the production of matrix-degrading enzymes (Müller-Ladner *et al.* 1996).

The results of this work also show that in bGH Tg cartilage the: (a) deorganization of zonal structure and (b) profound alterations in chondrocyte growth/differentiation processes, are consistent with a severe osteoarthritis. Systemically and locally produced growth factors and hormones regulate cartilage metabolism. Alterations in levels of these factors or in their activity can influence the pathogenesis or cartilage destruction in

arthritic joints (Verschure *et al.* 1996b). Cartilage alterations in bGH Tg mice can be interpreted in this sense. The specific role of GH in cartilage is unknown although GH receptors are present in chondrocytes (Nilsson *et al.* 1990). In addition, it has been shown that IGF-I is the most important growth factor that balances chondrocytes proteoglycan synthesis and catabolism (Verschure *et al.* 1996a, Van den Berg 1997). Clear zonal correlation has been detected between IGF-I receptor and proteoglycan synthesis which is lost in osteoarthritis (Verschure *et al.* 1996b). Moreover, increased levels of IGF-I and IGF-binding protein-3 have been determined in synovial fluids of patients with rheumatoid arthritis (Matsumoto *et al.* 1998). Thus, the cartilage damage of bGH Tg mice is likely produced by the effect of sustained high GH/IGF-I levels. As cartilage destruction is a major complication of arthritic processes, bGH Tg mice constitute a suitable model with which to improve our understanding of the biology and pathology of articular cartilage.

The serological determinations performed in this work show that bGH Tg mice develop an autoimmune-like process, that this immune disorder appears in adult animals and is more relevant in the females. The mechanism by which autoimmune disorders are generated is not well understood, although several hypotheses have been proposed (Van Noort & Amor 1998). In fact, it is likely that most autoimmune diseases are multifactorial in origin. The results suggest that the immune disorder observed in bGH Tg mice could be mediated by the effect of sustained high GH/IGF-I levels. The expression of GH receptor and IGF-I receptor in immune cell system has been reported as well as the induction of lymphopoiesis and haematopoiesis *in vivo* and *in vitro* (Kooijman *et al.* 1995, Gagnerault *et al.* 1996, Clark 1997, Mello-Coelho *et al.* 1998). Thus, it can be suggested that sustained GH/IGF-I levels are involved in an increased lymphoiesis and cell survival, thereby suppressing apoptosis of self-reactive lymphocytes. In this way, chronic high GH/IGF-I levels in Tg mice would produce an autoimmune-like pathology that closely resembles that described in MRL mice (Cohen & Eisenberg 1991). Furthermore, recent reports showing the anti-apoptotic effect of GH and IGF-I support this notion (Kulik & Weber 1998, Haeflner *et al.* 1999) and would also explain the subclinical activation of lupus nephritis reported by GH administration (Yap *et al.* 1998). However, further works need to be performed in order to understand the mechanism underlying the autoimmune-like process displayed in Tg mice.

In summary, our results show that bGH Tg mice develop a spontaneous autoimmune-like disorder accompanied by profound alterations in joints. Whether both phenomena are related or not, and the specific role of GH/IGF-I, remain to be elucidated. Given that cartilage destruction is a major complication of osteoarthritis and rheumatoid arthritis we therefore propose that bGH Tg mice are a suitable and reliable model for investigating

GH/IGF-I-driven processes in joint tissues and in immune cells and the pathophysiology of joints.

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