The Effects of Gonadotropin Treatment on the Immunological Features of Male Patients with Idiopathic Hypogonadotropic Hypogonadism

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ABSTRACT
There is a significant line of evidence for a role of androgens in the modulation of the immune system. However, little is known about immunological features of male patients with idiopathic hypogonadotropic hypogonadism (IHH) and the potential effects of gonadotropin treatment. Thus, the objective of this study was to evaluate the levels of selected soluble immune parameters [IgA, IgG, IgM, C3c, C4, interleukin-2 (IL-2), and IL-4], the CD4+/CD8+ ratio, and counts of total lymphocyte and some subpopulation of lymphocytes (CD3+, CD4+, CD8+, and CD19+ cells) before and after gonadotropin treatment in men with IHH. Twenty-nine IHH patients and 19 age-matched healthy controls were included in the study. The patients were treated with human menopausal gonadotropin/hCG for 6 months.

The pretreatment levels of serum Igs, C3c, IL-2, and IL-4 in the patient group were significantly higher than those in the controls (P < 0.001 for all). After treatment, all Igs (P < 0.001), C3c (P < 0.01), and IL-2 and IL-4 levels (P < 0.005) were decreased significantly compared to pretreatment levels. Pretreatment lymphocyte counts (P < 0.05); the percentages of CD3+ cells (P < 0.001), CD4+ cells (P < 0.001), and CD19+ cells (P < 0.001); and the CD4+/CD8+ ratio in the patient group were significantly higher (P < 0.05) than those in the controls. After treatment, the lymphocyte count (P < 0.001); CD3+ (P < 0.01), CD4+ (P < 0.001), and CD19+ (P < 0.005) cells; and the CD4+/CD8+ ratio (P < 0.001) were decreased, but CD8+ cells were increased significantly (P < 0.001).

In summary, lack of testosterone action results in the enhancement of cellular and humoral immunity. The results of this study allowed us to conclude that testosterone deficiency affects both cell-mediated and humoral immunity, and these may be modulated with gonadotropin therapy in male patients with IHH.

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The immune system exhibits different features in terms of gender. In general, women have enhanced immunological activity, including higher plasma IgG, IgM, interleukin-1 (IL-1), and IL-4 levels; a higher CD4+/CD8+ ratio; and a lower number of CD8+ cells (1, 2).

It has long been known that many diseases believed to be autoimmune in origin are more prevalent in females than in males in both humans and experimental animal models (3, 4). It is also known that autoimmune and rheumatic diseases are not uncommon in Klinefelter’s syndrome (KS) (5, 6). This diversity of the prevalence of autoimmune disorders is partly attributed to the differential actions of sex steroid hormones on the immune system (7, 8).

The effects of estrogens and androgens on the immune system differ (1). Castration of male mice results in increased B cells in the periphery (9), thymic enlargement (10, 11), and a decrease in thymic CD3+ cells (12). Androgens exert an inhibitory effect on the thymus and cause thymic involution (13) and increase CD8+ activity in mice (14). Moreover, androgens inhibit B cell hyperactivity and Ig production (15), and decrease CD4+ cells in humans (16). Furthermore, IL-2 and IL-4 levels are higher in mice with testicular feminization and in those with androgen resistance, respectively (11, 17).

On the other hand, lower numbers of CD3+ and CD8+ cells and increased CD4+/CD8+ ratio, which were normalized with androgen replacement therapy, have been reported in patients with KS who have rheumatic diseases (18, 19). However, little is known about the immunological features of men with idiopathic hypogonadotropic hypogonadism (IHH) and the effects of gonadotropin treatment on these features. Thus, the objective of this study was to measure the levels of selected soluble immune parameters (IgA, IgG, IgM, C3c, C4, IL-2, and IL-4), the CD4+/CD8+ ratio, and counts of total lymphocyte and some subpopulations of lymphocytes (CD3+, CD4+, CD8+, and CD19+ cells) and to evaluate the effects of gonadotropin treatment on these parameters in men with IHH.

Subjects and Methods

Subjects
Twenty-nine untreated men with IHH (mean age, 21.7 ± 1.49 yr; range, 20–23 yr) and 19 age-matched healthy men (mean age, 21.7 ± 1.50 yr; range, 20–23 yr) were enrolled in the study. The diagnosis of IHH was based on failure to undergo spontaneous puberty before 18 yr of age and was confirmed by a decreased serum testosterone concentration below normal range for adults, FSH and LH levels within or below the normal range, absence of a pituitary or hypothalamic mass lesion on computerized tomography or magnetic resonance imaging, presence of a gonadotropin response to repetitive doses of GnRH, normal smell test, and normal karyotypes (46,XY). None of the patients had hyposmia,
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anemia, or a family history of IHH. All patients had scrotal tests. All patients and control subjects were informed about the aim and procedures of the study and gave their consent. The study was approved by the ethical committee of Gulhane School of Medicine.

**Study design**

Patients were treated with hCG (Profasi HP 2000, Serona SA, Aubonne, Switzerland; containing 2000 IU hCG) and human postmenopausal gonadotropin (Pergonal, Serona SA; containing 75 IU FSH and 75 IU LH) three times a week for 6 months.

Blood samples were collected from patients and controls between 0800–0830 h after overnight fasting. Posttreatment blood samples from the patient group were drawn 2 days after injection of human menopausal gonadotropin/hCG.

**Measurements**

Complete blood count analyses were performed with automatic hemocounter (Cell-Dyn 1700, Abbott, Santa Clara, CA). Serum FSH, LH, and PRL were measured by immunoradiometric assay with reagents from Behring (BN 100, Behringwerke AG, Marburg, Germany). Serum testosterone (FT) was determined by a solid phase 125I RIA with reagents from Radim Techland (Angleur, Belgium). The intra- and interassay coefficients of variation (CVs) were 4.4% and 6.0% for FSH, 4.8% and 5.4% for LH, and 4.6 and 6.0% for PRL, respectively. Serum free testosterone (FT) was determined by a solid phase 125I RIA with reagents from Diagnostic Products (Los Angeles, CA). The intra- and interassay CVs were 3.8% and 4.2% for FT, respectively. Serum sex hormone-binding globulin (SHBG) was measured by RIA with reagents from Radim Techland (Angleur, Belgium). The intra- and interassay CVs were 2.4% and 2.9% for SHBG, respectively. The normal ranges in our laboratory are less than 15 IU/L for FSH, less than 20 IU/L for LH, 15–45 pg/mL for FT, and 9–55 nmol/L for SHBG. The upper limit for PRL is 12 µg/L consecutively.

Lymphocyte subpopulations were assessed by flow cytometry with monoclonal antibodies, reagents, and instrument (FASCalibur) from Becton Dickinson and Co. (San Jose, CA) within 2 h after blood sample collection. The intra- and interassay CVs were 3.2% and 2.47% for CD4⁺ cells, 3.6% and 3.28% for CD8⁺ cells, 1.7% and 1.8% for CD3⁺ cells, 21.5% and 10.59% for CD19⁺ cells, and .2% and 4.80% for the CD4⁺/CD8⁺ ratio, respectively.

Serum IgG, C3c, and C4 levels were determined by nephelometry with reagents from Behring (BN 100, Behringwerke AG, Marburg, Germany). The intra- and interassay CVs were 1.7% and 1.0% for IgG, 2.8% and 1.1% for IgA, 1.7% and 1.4% for IgM, 2.6% and 1.9% for C3c, and 2.6% and 3.3% for C4, respectively.

Serum IL levels were measured by enzyme immunoasay with reagents from BioSource International (Camarillo, CA).

**Statistical analysis**

All results are given as the mean ± sd. Comparisons between the patient and the controls were performed with unpaired, and comparisons between pre- and posttreatment values were performed with paired Student’s *t* test. *P* < 0.05 was considered statistically significant.

**Results**

The mean levels of hormones and pre- and posttreatment immunological parameters are given in Table 1. As seen in Table 1, the mean FSH, LH, and FT levels were significantly lower and SHBG levels were significantly higher in the patient group than in controls. PRL levels were not different however.

After gonadotropin treatment, mean FT levels were significantly increased. SHBG and PRL levels did not change significantly.

Baseline IgG, IgA, and IgM levels were significantly higher in the patient group than in controls. After gonadotropin treatment, Ig levels were decreased significantly.

Compared with the control group, pretreatment total lymphocyte counts; the percentages of the CD3⁺, CD4⁺, and CD19⁺ lymphocyte counts; and the CD4⁺/CD8⁺ ratio were significantly higher in the patient group. With regard to the percentage of the CD8⁺ lymphocyte counts, the difference was not significant. After gonadotropin treatment, total lymphocyte count; the percentages of the CD3⁺, CD4⁺, and CD19⁺ counts; and the CD4⁺/CD8⁺ ratio were decreased significantly, but the percentage of CD8⁺ increased significantly.

For ILs, the mean IL-2 and IL-4 levels were significantly higher in the patient group than in the control group (*P* < 0.001 and *P* < 0.001, respectively). IL-2 and IL-4 levels in the patients were significantly decreased after gonadotropin treatment (*P* < 0.005 and *P* < 0.005, respectively).

**TABLE 1. Hormonal and immunological parameter of the patients and the controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>Controls (mean ± sd)</th>
<th>Baseline vs. controls</th>
<th>Pre vs. posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/mL)</td>
<td>0.81 ± 0.32</td>
<td>1.29 ± 1.99</td>
<td>3.74 ± 1.65</td>
<td>&lt;0.001 NS</td>
<td>NS</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>1.28 ± 1.31</td>
<td>1.46 ± 1.56</td>
<td>4.08 ± 1.62</td>
<td>&lt;0.001 NS</td>
<td>NS</td>
</tr>
<tr>
<td>FT (pg/mL)</td>
<td>1.68 ± 0.91</td>
<td>23.03 ± 5.01</td>
<td>28.96 ± 10.26</td>
<td>&lt;0.001 &lt;0.001</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>41.34 ± 13.98</td>
<td>35.79 ± 9.07</td>
<td>28.64 ± 5.47</td>
<td>&lt;0.001 NS</td>
<td>NS</td>
</tr>
<tr>
<td>PRL (µg/L)</td>
<td>7.06 ± 4.31</td>
<td>7.31 ± 4.76</td>
<td>6.32 ± 3.32</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>18.67 ± 4.54</td>
<td>14.04 ± 3.48</td>
<td>14.93 ± 2.50</td>
<td>&lt;0.001 NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgA (g/L)</td>
<td>3.46 ± 0.85</td>
<td>2.05 ± 0.80</td>
<td>1.96 ± 0.67</td>
<td>&lt;0.001 NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgM (g/L)</td>
<td>3.67 ± 0.69</td>
<td>2.10 ± 0.88</td>
<td>1.79 ± 0.47</td>
<td>&lt;0.001 NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C3c (g/L)</td>
<td>0.94 ± 0.20</td>
<td>0.81 ± 0.30</td>
<td>0.75 ± 0.14</td>
<td>&lt;0.001 NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C4 (g/L)</td>
<td>0.29 ± 0.09</td>
<td>0.22 ± 0.6</td>
<td>0.27 ± 0.08</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lymphocyte count (mm³)</td>
<td>2101.1 ± 411.94</td>
<td>1700.6 ± 292.3</td>
<td>1836.3 ± 287.5</td>
<td>&lt;0.001 &lt;0.001</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>CD4⁺ (%)</td>
<td>45.09 ± 2.78</td>
<td>36.35 ± 3.00</td>
<td>41.60 ± 3.55</td>
<td>&lt;0.001 &lt;0.001</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>CD8⁺ (%)</td>
<td>25.37 ± 2.87</td>
<td>30.14 ± 3.15</td>
<td>25.55 ± 2.93</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD3⁺ (%)</td>
<td>71.76 ± 3.52</td>
<td>66.95 ± 3.14</td>
<td>67.65 ± 3.16</td>
<td>&lt;0.001 &lt;0.01</td>
<td>&lt;0.001 &lt;0.01</td>
</tr>
<tr>
<td>CD19⁺ (%)</td>
<td>12.62 ± 1.77</td>
<td>10.74 ± 1.62</td>
<td>11.84 ± 2.37</td>
<td>&lt;0.005 &lt;0.005</td>
<td>&lt;0.005 &lt;0.005</td>
</tr>
<tr>
<td>CD4⁺/CD8⁺</td>
<td>1.83 ± 0.23</td>
<td>1.22 ± 0.21</td>
<td>1.65 ± 0.25</td>
<td>&lt;0.05 &lt;0.001</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>20.70 ± 9.64</td>
<td>13.65 ± 5.09</td>
<td>12.70 ± 3.55</td>
<td>&lt;0.001 &lt;0.001</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>12.78 ± 8.78</td>
<td>7.13 ± 4.76</td>
<td>4.91 ± 2.42</td>
<td>&lt;0.001 &lt;0.005</td>
<td>&lt;0.001 &lt;0.005</td>
</tr>
</tbody>
</table>

FT, Free testosterone; TLC, total lymphocyte count; P1, IHH vs. controls; P2, pretreatment vs. posttreatment.
C3c levels were significantly higher ($P < 0.01$) before treatment compared with the control values, whereas C4 levels were not significantly higher. After gonadotropin treatment, both C3c and C4 levels were decreased significantly ($P < 0.001$ and $P < 0.01$, respectively).

**Discussion**

It has long been known that a number of autoimmune and rheumatic diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren’s syndrome (SS), autoimmune thyroiditis, and primary biliary cirrhosis, are more prevalent in women than in men, as are SLE and some other autoimmune diseases in KS (3–5).

Many abnormalities in the metabolism of estrogen and androgen in SLE (20) and reduced serum concentrations of androgens in RA, SS, and adjuvant-induced arthritis have been reported (19–21). Based on these observations, hormonal modulation of rheumatic diseases has been investigated. Androgen therapy has been used to effectively ameliorate SS, SLE, thyroiditis, and polyarthritis in animal models and to decrease various signs and symptoms of SLE, SS, and RA in humans (2, 22–24). The effects of testosterone treatment are related not only to serum testosterone levels, but also to other factors, such as androgen metabolism, androgen receptor expression and sensitivity, androgen/estrogen ratio, the effect of the cytokines on sex steroid metabolism, the emotional status of the patient, etc.

Ig levels tend to differ between women and men. Elevated levels of IgG and IgM have been reported in KS (25). Butcher et al. studied IgG, IgA, and IgM levels in 315 adults and 503 children and reported that female have increased IgG and IgA levels, but these levels were not significantly higher than those in males. However, IgM level was significantly higher in females than males in both adults and children (26). Lichtman et al. reported that women have higher levels of plasma IgM and IgG than males (27). When testosterone was added to the culture medium of peripheral mononucleated cells from patients with SLE, anti-DNA antibody and total IgG production had decreased (15). In our study IgG, IgA, and IgM levels were higher than the control values and decreased to normal levels after treatment in 29 male patients with IHH. It may be derived that Ig production in untreated IHH is enhanced, similar to that in women, and may be inhibited by the treatment. Decreases observed in Igs after treatment may be explained by the effects of testosterone, which is shown to inhibit B cell hyperactivity and *in vitro* Ig production (15) and to increase CD8⁺ activity (14).

A higher CD4⁺/CD8⁺ ratio is generally seen in females and patients with KS due to lower circulating CD8⁺ T lymphocytes (2, 18, 28). Androgens decrease CD4⁺ T lymphocytes and the CD5⁺/CD8⁺ ratio in humans (16) and increase the activity and number of CD8⁺ cells *in vivo* and *in vitro* (7, 18, 22). On the other hand, in five male patients with KS (three of them associated with SS and two with SLE) lower pretreatment CD3⁺ and CD8⁺ T cell counts and increased CD4⁺/CD8⁺ ratio were normalized with androgen replacement treatment (18). In another study, the relatively low number of peripheral CD8⁺ T cells increased and the high pretreatment CD4⁺/CD8⁺ ratio decreased after 3–6 months of testosterone treatment in male patients with RA who had low testosterone levels (22). Kiess et al. (29) evaluated lymphocyte distribution in patients with IHH before and after treatment. Contrary to our findings, they reported that the percentage of CD3⁺ and CD8⁺ cells, the CD4⁺/CD8⁺ ratio, and the natural killer cell activity of peripheral mononuclear cells did not differ between patients and controls and did not change after treatment.

CD19⁺ cells represent mature B cells, whereas CD3⁻ cells are mainly mature T cells. The posttreatment decrease observed in CD19⁺ cells is consistent with decreased levels of Igs. Pretreatment high CD3⁺ cell numbers and a decrease after gonadotropin treatment may not be considered to be consistent with the finding of Olsen et al., who observed fewer CD3⁺ in thymus of castrate male mice than in intact controls (12). The changes observed in lymphocyte subpopulations in this study suggest that gonadotropin treatment may have a net negative effect on Ig synthesis of B lymphocytes. However, this suggestion needs further explanation. Although they have been detected in pre-B (Raji) cells and in some B cell lineages, it is believed that neither peripheral B lymphocytes nor bone marrow B cell precursors do not express androgen receptors (AR) (1). To date there are studies, which are mostly experimental, suggesting that testosterone has effects on B cell lymphocyte populations (9). Castration of male mice results in an expansion of the pre-B cell population in bone marrow and increased mature B lymphocytes in the periphery (9, 30). Androgen replacement reverses postcastrational changes (31). *In vitro* testosterone suppresses antibody production by B cells in a clinical study by Naoko et al. (15).

It is tempting to postulate that the Ig-lowering effect of hormone replacement therapy may be due to regulation of T cell functions, because other data suggest that T cells are an important component of hormonal regulating pathways (1, 15). However, AR expression could not be demonstrated in peripheral T cells (32).

On the other hand, it has been known for nearly 100 yr that the thymus is sensitive to the actions of androgens (13). Castration causes thymic enlargement (10, 33). Mice with testicular feminization also exhibit thymic enlargement (11). Physiological thymic involution may be reversed by castration and reinstituted by testosterone replacement in animals (12, 33). Taken together, it may be postulated that the thymus of hypogonadal males might not have completed its involution and may constitute the mainstay of our data. However, the nature of the cells in the thymus must be ascertained. Many studies showed that thymocytes express AR and are potential targets for androgens (34–36). AR expression at low levels in thymic epithelial and stromal cells has been reported (34, 37). Olsen et al. (38) recently demonstrated that androgens accelerate thymocyte apoptosis. The effects of androgen on T cells may be indirect and mediated by immunomodulatory signals from epithelial cells and macrophages (39). Olsen recently suggested that androgens act on intrathymic stages of T cell development, ultimately changing the characteristics and effector functions of the expressed T cell repertoire (40). Therefore, the expression of AR on splenic, thymic, bone marrow, epithelial, and stromal cells and on...
thymus activity should be determined in this young hypogonadal male population before making a rational suggestion about the cellular basis relevant to our data. The relationship between autoimmune disorders and sex steroid receptor defects is another matter of concern. Numerous defects in ARs have been reported in various tissues (41).

The interaction between ILs and gonadal hormones has been documented (11, 42, 43). Batticane et al. demonstrated that induction of lymphocyte activation in vitro with LHRH is accompanied by a specific increase in IL-2 receptor-positive cells (44). IL-2 production is under the control of testosterone in male mice and concanavalin A-stimulated spleen cells from female mice produce more IL-2 than males (42). The IL-2 level is 4 times greater in mice with testicular feminization than in normal mice (11), and castration leads to an increase in IL-2 levels in normal mice (3, 45). As for IL-4, it was shown that dihydrotestosterone exerts a depressive effect on the production of IL-4 by activated murine T cells (46). Lymphocytes and splenic cells from female mice release more IL-4 (1). A high level of IL-4 production is reported in androgen resistance (17) and during pregnancy in mice (47). The effect of castration on IL-4 could not be demonstrated by Golding et al. (30). IL-2 is a mediator produced chiefly by the Th1 subset of helper/inducer T cells and stimulates other T cells. IL-2 is produced by Th2 cells, targeted to T and B cells, and induces differentiation and IgG and IgE production (48).

In our study, IL-2 and IL-4 levels before treatment were in androgen replacement therapy. In summary, lack of testosterone action causes enhancement of the immune system. The results of our study allowed us to conclude that testosterone deficiency interferes with both cell-mediated and humoral immunity, and these may be modulated by gonadotropin therapy in male patients with IHH.

Our findings at the baseline (high levels of Igs, IL-2, IL-4, and C3c; increased numbers of peripheral CD4+, CD3+, CD19+ cells; and elevated CD4+/CD8+ ratio) may suggest that immune system in IHH appears to be enhanced by the action of testosterone deficiency and appear to be consistent with immunological changes observed in castrate mice. Post-treatment data (decreased Igs, IL-2, IL-4, and C3c levels; reduced numbers of CD3+, CD4+, and CD19+ cells; and decreased CD4+/CD8+ ratio) may be explained by immunosuppressive androgenic influences after gonadotropin treatment.

To our knowledge, this is a novel study designed to evaluate some immunological parameters in IHH before and after hormone replacement therapy. In summary, lack of testosterone action causes enhancement of the immune system. The results of our study allowed us to conclude that testosterone deficiency interferes with both cell-mediated and humoral immunity, and these may be modulated by gonadotropin therapy in male patients with IHH.

References


