Lymphocyte subset pattern in acromegaly

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ABSTRACT. Immune function in acromegalic patients has been poorly investigated. The aim of this study was to evaluate the main surface antigen clusters of circulating lymphocytes in acromegaly. One hundred patients with active acromegaly (55 women and 45 men, aged 20-70 yr) and 200 healthy subjects sex- and age-matched with the patients (110 women and 90 men, aged 20-70 yr) were enrolled in this study. All patients and controls were born and live in Southern Italy. No patient had received octreotide, bromocriptine or corticosteroids for at least 3 months before entering the study. The analysis of lymphocyte subset pattern was performed by flow cytometry and fluorescein isothiocyanate or phycoerythrin directly conjugated monoclonal antibodies specific for the cell surface antigen clusters (CD) representing T-cell population as a whole (CD3), T helpers (CD4), T suppressors (CD8), natural killer cells (CD16) and B-cell population as a whole (CD19). Acromegalics had significantly increased levels of CD3 (67.1±7.2 *vs* 64.3±8.8%; *p*=0.03) and CD4 (37.8±3.5 *vs* 36.4±4.3%; *p*=0.004) and decreased levels of CD8 (31.4±3.3 *vs* 33.7±8.2%; *p*<0.01) and CD19 (12.1±3.1 *vs* 15.2±5.1; *p*=0.01) without age-difference. The results of the current study demonstrate an increase in T-cell activity together with a decrease in B-cell activity in a very large series of patients with active acromegaly. These data further support the existence of abnormalities of the immune system in patients with chronic GH/IGF-I excess.

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INTRODUCTION

In acromegaly, various tumors have been reported to occur with a greater than expected incidence (1-3). In particular, prevalence of tumors of the gastrointestinal tract was suggested to be higher in acromegalics than in the healthy population (4-8), although negative data have also been reported recently (9). GH/IGF-I axis has long been supposed to play a major role in immuno-modulation: in rats hypothalamic damage leads to abrogation of natural killer cell activity (10), whereas fetal hypophysectomy in mice results in thymic atrophy and immunodeficiency (11). Moreover, specific GH receptors have been found on peripheral mononuclear cells (12); GH modulates lymphoproliferation *in vitro* (13-15) and is considered a minor growth factor for normal lymphocyte *in vivo* (16). On the other hand, GH-deficient humans are not immunodeficient (17) and immune function in GH-treated children has been reported to be normal (18), increased (19) or decreased (20). Despite this conflicting evidence on the association between GH secretory status and modulation of immune response, immune function in acromegalic patients has been poorly investigated.

The aim of this study was to evaluate the lymphocyte subset pattern in a large series of consecutive active acromegalic patients, and compare it to a consistent control group of Caucasian healthy sex- and age-matched individuals, living in Southern Italy.

PATIENTS AND METHODS

Subjects

One hundred acromegalic patients with active disease (55 women and 45 men, aged 20-70 yr) entered this study. The diagnosis of acromegaly was based on clinical, hormonal and radiological features, as elsewhere reported (21, 22). GH was 31±8.5 µg/l not suppressed below 1 µg/l after oral administration of 75 g glucose, IGF-I was 780.5±66 µg/l. No patient had received octreotide or bromo-

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criptine for at least 3 months before entering the study. L-thyroxine, given at the dose of 75-125 µg/day for goiter to 63 patients, was withdrawn 15-30 days before entering the study. Two-hundred healthy subjects of the medical and paramedical staff of the Departments of Molecular and Clinical Endocrinology and Oncology of Federico II University and of the Unit of Oncology and of Laboratory of the Cardarelli Hospital in Naples, sex- and age-matched with patients, served as controls (110 women and 90 men, aged 20-70 yr). All patients and controls were born and lived in Southern Italy. The exclusion criteria for acromegalics and controls were: age over 70 yr, previous benign and malignant tumors, anemia, previous radiation and corticosteroid therapy performed 3 months before entering the study. All patients and controls gave their informed consent orally in the presence of a third person.

Methods

The analysis of lymphocyte subset pattern was performed by flow cytometry using aliquots of whole fresh blood collected from an antecubital vein and fluorescein isothiocyanate (FITC) or phycoerythrin (PE) directly conjugated monoclonal antibodies (MoAbs) specific for the cell surface antigen clusters (CD) 3, 4, 8, 16 and 19 (Becton Dickinson Monoclonal Center Inc., Mountain View, CA, U.S.A.). Lymphocyte gated on light scatter were analyzed for fluorescence intensity using FACScan (Becton Dickinson) acquiring 5000 events for at least each sample.

Assays

Serum GH levels were measured by IRMA (HGH-CTK-IRMA Sorin, Saluggia, Italy). The sensitivity of the assay was 0.2 µg/l; 1 µg/l corresponds to 2.5 mU/l. The intra- and inter-assay coefficients of variation (CV) were 4.5 and 7.9%, respectively. Plasma IGF-I was measured by IRMA after ethanol extraction using DSL kits (Webster, Tx, U.S.A.). The sensitivity of the assay was 0.8 µg/l. The intra-assay CV were 3.4, 3.0 and 1.5% for the low, medium and high points on the standard curve, respectively. The inter-assay CV were 8.2, 1.5 and 3.7% for the low, medium and high points on the standard curve. Fasting GH levels were considered above the normal range when >2.5 µg/l. In our laboratories the normal IGF-I range was 110-450 µg/l in ≤40, 100-300 µg/l in 41-59, and 78-258 µg/l in ≥60 yr old subjects.

Statistical analysis

Statistical analysis was performed by ANOVA. Significance was set at the 5%. Data are reported as Mean±SD.

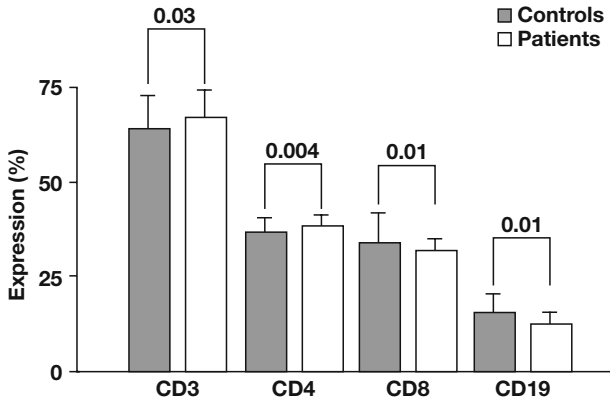

Fig. 1 - *Lymphocyte whole population (CD3), T helpers cells (CD4), T suppressor cells (CD8), and B-cell population as a whole (CD19) in 100 patients with acromegaly and 200 controls.*

RESULTS

In acromegalic patients, the expression of the whole lymphocyte population (CD3) and of T helpers cells (CD4) was significantly increased, when compared to control subjects (Fig. 1). By contrast, the expression of T suppressor cells (CD8), and B- cell population as a whole (CD19) was significantly decreased (Fig. 1). No significant difference was found between acromegalics and controls in the expression of natural killer cells, represented by the natural killers (CD16) expressing cells (9.9±4.9 *vs* 10.2±3.3%). In addition, a significant decrease of the ratio between CD4 and CD8 was observed in acromegalic patients compared to controls (1.2±0.5 *vs* 1.5±0.5; $p<0.001$). No difference in the expression of CD3, CD4, CD8, CD16 and CD19 was found among young (age below 40 yr), middle-aged (40-60 yr) or elderly (>60 yr) patients with acromegaly (Fig. 2).

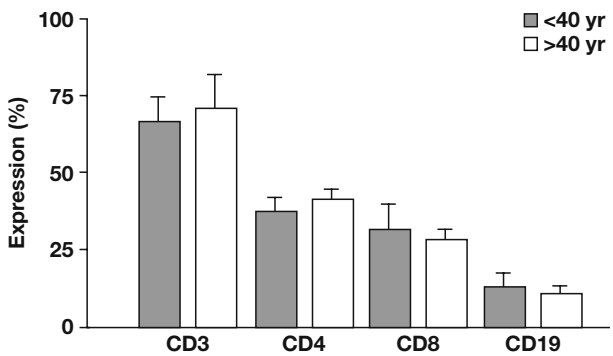

Fig. 2 - *Lymphocyte whole population (CD3), T helpers cells (CD4), T suppressor cells (CD8), and B-cell population as a whole (CD19) in 100 patients with acromegaly grouped on the basis of age below (no.=38) or above 40 yr (no.=62).*

DISCUSSION

The results of the current study demonstrate a decrease of T-and increase of B-cell lines in patients with active acromegaly as compared to sex- and age-matched controls. The changes in the lymphocyte subset pattern were independent from the patients' age. GH has been proposed to be an essential hormone for the development, maintenance and regulation of immune function (23, 24): it is necessary to maintain lymphatic tissues populated with lymphocytes since the removal of GH results in thymus atrophy and secondary lymphoid tissues (25-28). GH has been shown to induce differentiation both of thymocyte precursors into helper T-cells (29) and of stem cells into granulocytes (30). Moreover, GH can increase *in vitro* development of cytotoxic T lymphocytes in mixed lymphocyte cultures (24) and activate macrophages (31). On the other hand, GH may enhance the *in vitro* proliferation of virus-injected erythroleukemia cells (14) and of certain types of transformed cells (13-15). In addition, circulating GH and IGF-I have been reported to promote leukemia blast cell replication *in vivo* (32). Over-expression of heterologous or homologous GH in transgenic mice was shown to induce significant stimulation of some parameters of immune function (32). In fact, in metallothionein I (MT)-bGH transgenic mice with high peripheral levels of bovine GH, the absolute weight of the thymus and the spleen was significantly increased and the mitogenic responses of splenocytes to concanavalin A (ConA), lipopolysaccharide (LPS) and phytohemagglutinin (PHA) were enhanced, as compared to age-matched normal animals (32).

Although the neoplastic risk has been claimed to be increased in acromegaly (1-9), data regarding immune functions in patients with acromegaly are currently limited. In a study reported by others (7), the lymphocyte subset pattern in the colonic *lamina propria* of 34 acromegalics showed a significant decrease of B-cells (CD19, CD20), CD16, γ/δ ratio, considered as index of intraepitelial cytotoxic activity (33), and an increase of T-cells as a whole (CD3), when compared to controls. Interestingly, the changes of the lymphocyte subset pattern were found in patients with active disease while those with inactive disease had a pattern similar to healthy subjects. Since the study was performed at the mucosal level of the colonic *lamina propria* close to the polyp lesion, the decrease of the helper-inducer ($CD4^+/leu8^-$) lymphocytes suggested an impairment of the immunoglobulin synthesis mediated by the above-mentioned T-cells at mucosal level (34). In contrast, the increase of the helper-suppressor ($CD4^+/leu8^+$) lymphocytes, supposed to regulate the cell-mediated immune response at mucosal locoregional site, could be caused by the colonic polyp itself (7). However, patients with and without polyps had a similar lymphocyte subset pattern, thus excluding a pathogenetic role for colonic polyps in altering the lymphocyte subset pattern (7). The existence of an altered immune function in acromegaly was also reported in a small series of acromegalic patients and controls: while natural killer cell activity, serum concentrations of immunoglobulins (IgG, IgM, IgA) and metabolic burst activity were within the normal range in both groups, a significantly enhanced phagocytic activity was observed in patients with acromegaly. Kotzmann *et al.* (35) reported also that surface markers on T lymphocytes (CD3, CD4, CD8), B lymphocytes (CD19) and natural killer cells (CD16/56) were normal in both groups, but $CD4^+$ and $CD8^+$ cells showed a significantly higher expression of transferrin receptors in acromegalic patients, indicating enhanced T-cell activity. In our current series of patients with acromegaly, T-cell activity, measured as CD3 and CD4, was also shown to be enhanced.

Furthermore, aging has been shown to be accompanied by various changes in the lymphocyte subset distribution. In fact, a significant increase in both the absolute counts and the proportions of CD3 and CD57 was found with age, whereas the cytolytic T-cell population showed less change with age (36). In addition, soluble interleukin-2 receptor levels were found to increase significantly with age and correlated with certain natural killer cell subsets (36). The functions of these changes are still unclear but the expansion of some lymphocyte subsets in the elderly was suggested to represent a remodeling of the immune system with ageing, with an increase in non-MHC (major histocompatibility complex)-restricted cells likely to compensate the previously reported decline in T- and B-cells (36). This decline in T- and B-cells was also found in elderly acromegalics as compared to young and middle-aged patients, suggesting the persistence of this remodeling mechanism in patients with chronic GH/IGF-I hypersecretion.

The results of the current study demonstrate an increase in T-cell activity together with a decrease in B-cell activity in a very large series of patients with active acromegaly. These data further support the existence of abnormalities of the immune system in patients with chronic GH/IGF-I excess.

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