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REVIEW

Low level IGF-1 and common variable immune deficiency: an unusual combination

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ABSTRACT

A relation between growth hormone (GH) deficiency and immunoglobulin deficiency has been suggested previously in a few cases. We describe a patient with an insulin-like growth factor 1 (IGF-1) deficiency and common variable immune deficiency and briefly review earlier publications on the possible interaction between IGF-1 and the immune system.

IGF-1 is the downstream mediator of GH. In this patient, GH and IGF-1 levels were both low. The GH response to a GH-releasing hormone test was normal whereas no subsequent IGF-1 response was seen. In our cohort of 14 patients with hypogammaglobulinaemia, two turned out to have slightly decreased IGF-1 serum levels and one patient with a thymoma had an increased IGF-1 level.

Even though IGF-1 may be connected to B lymphocyte differentiation, in this patient we hypothesise there is a common impairment in the IGF-1 and IgG pathways.

KEYWORDS

CVID, hypogammaglobulinaemia, IGF-1 deficiency

INTRODUCTION

Heterogeneous syndrome common variable immunodeficiency (CVID, OMIM 240500) is characterised by hypogammaglobulinaemia, absent or poor vaccination response and recurrent bacterial infections. Among CVID patients a higher incidence of autoimmune diseases is found and also certain malignancies. Typically, levels of immunoglobulin G and A (IgG, IgA) are low in CVID patients; however the IgM level can also be deficient. The prevalence varies from 1:25,000 to 100,000. Some studies suggest a higher prevalence among North-Europeans. It remains unclear whether CVID is due to a primary B cell defect or deficient T-B cell interaction.[1,2] Genetically, CVID seems to be a heterogeneous entity. Only recently, four genes have been recognised to be associated with or causative for (in case of homozygote mutation) CVID. Mutations in the TNF-receptor family member transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), which mediates isotype switching in B cells, explain up to 10% of CVID cases. The other known molecular defects associated with CVID are mutations in the inducible co-stimulatory (ICOS) receptor, the BAFF receptor which together with BAFF is essential for B cell survival in the periphery, and CD19, a cell surface molecule expressed on B cells and follicular dendritic cells.[3-5] Apart from CVID, these mutations result in a further normal phenotype.

Insulin-like growth factor 1 (IGF-1) is an anabolic hormone and the key mediator of growth hormone (GH). Most mesenchymal cell types synthesise IGF-1. Primary IGF-1 deficiency can be described as a decrease in IGF-1 production without a concomitant impairment in GH secretion.[6] Known molecular defects resulting in primary IGF-1 deficiency are mutations or deletions in the GH gene, post GH receptor defects (JAK/STAT), mutations in the acid label subunit (ALS) gene and mutations or deletions in the IGF-1 gene.[7] Regulation of IGF-1 occurs in two ways: synthesis in the liver and secretion into blood under control of GH and auto/paracrine synthesis in peripheral tissues,

controlled by GH and locally secreted factors. GH – both directly and through IGF-1 – has been implicated in T and B lymphocyte development and regulation in several studies.[8-13] *In vitro* experiments suggest a role for GH and IGF-1 in immunoglobulin production.[14-18] Recently, two patients diagnosed with both CVID and defects in the GH/IGF-1 axis have been described.[19,20] To our knowledge we present the first patient with CVID and a primary IGF-1 deficiency.

CASE REPORT

Medical history

A mentally retarded, Caucasian male was diagnosed with CVID at the age of 14 after years of persistent intestinal inflammation, recurrent pulmonary and middle ear infections, lymphadenopathies and multiple *S. aureus* infections, the last-mentioned being rather uncommon in CVID. Diagnosis was based on the following findings: (1) IgG and IgA deficiency (serum IgG 1 g/l; IgA 0.07 g/l) without gastrointestinal or renal loss of IgG/IgA, (2) B cells were found as well as normal B cell precursor counts in the bone marrow. Moreover, bone marrow lacked IgG/IgA positive plasma cells and small intestine biopsy showed loss of IgA and IgM positive cells. Ataxia telangiectasia and fragile X syndrome were ruled out by DNA tests.

The patient presented with growth retardation and underweight at a very young age. At the age of 3 a single small intestine biopsy was taken, showing villous atrophy. Although coeliac disease was never proven according to the current standard (three biopsies), a gluten-free diet was started which led to weight normalisation. During adolescence the patient's growth curve was increased to within the 'normal' range (at age 17, his height was 1.64 m., and at 22 years his height was 1.69 m.). In his teens he developed severe hearing impairment due to conduction problems, probably caused by recurrent infections.

At the age of 16 the patient presented with double-sided papiloedema and recurrent dacryocystitis. Four years later, during hospitalisation, a refractory pneumonia developed involving the entire left lung, resulting in atelectasis. Sputum contained *H. influenzae* and *S. pneumoniae*. Over the years, pulmonary infections were reported frequently, papiloedema remained and retinal vasculitis with neovascularisation developed. The background for these symptoms was not found. In 2002 and 2006 (age 35) enlarged lymph nodes in both groins developed as a consequence of clinical erysipelas. Pseudomonas was cultured on both occasions from lymph node biopsies. No signs of malignancy were found.

In the course of 2006 the patient's condition deteriorated gradually due to recurrent pneumonias for which he was hospitalised each time. His need for intravenous immunoglobulin (IVIg) increased drastically to up to 20 grams biweekly. In January 2007 the patient was hospitalised due to an aspiration pneumonia and within days transferred to the ICU where he was mechanically ventilated. During his stay at the ICU his condition deteriorated progressively and he succumbed at the age of 36 as the consequence of an irreversible septic shock.

Relatives

The patient's father suffers from diabetes mellitus type 2, hearing and vision problems and asthma. The physical features of father and son are very similar. One of the patient's two brothers suffered from type-1 diabetes and died at the age of 20 from fungal encephalitis and disseminated *Mycobacterium avium* infection; no underlying immune deficiency was detected at the time. The only sister was diagnosed with hypogammaglobulinaemia. She refused therapy (IVIg) and developed an aggressive form (treatment resistant) of non-Hodgkin's disease. She died at the age of 30. In the oldest brother, the diagnosis of mild phenotype CVID has been made as well. A cousin is also suffering from CVID in combination with refractory uveitis and hypothyroidism. The mother is healthy. There is no consanguinity.

Immuno-phenotyping and cell function

Flow cytometric analysis of peripheral blood revealed slightly increased absolute numbers of B cells, and decreased numbers of CD4+ T cells. More detailed typing of the B cells showed that 24% had a memory phenotype (CD27+) of which 3% were switched memory B cells (IgD-/CD27+). Somatic hypermutation had taken place in only 24.3% (normal 60%). CD40 and CD40 ligand were normally expressed (*table 1*), as were BAFF and TACI. Proliferation of phytohaemagglutinin (PHA) stimulated T cells was assayed in response to medium, CD2/CD28, IL-2, IL-7, or IL-15 using carboxyfluorescein diacetate succininyl ester (CFSE) labelling.[21] Proliferation was considerably reduced in response to CD2/CD28 and also reduced in response to IL-2, IL-7 and IL-15.

The bone marrow was practically devoid of plasma cells (0.1% compared with an average of 0.2 to 3.6%), whereas IgM plasma cells were found in normal quantities in lymph nodes. Moreover, Mott cells were detected in the May Grünwald-Giemsa stained inprints of the lymph node (*figure 1*). Mott cells are abnormal plasma cells defective in immunoglobulin secretion. They are filled with Russell bodies, vacuoles that contain immunoglobulins or immunoglobulin remnants. These cells can occasionally be found in multiple myeloma, trypanosomiasis and AIDS.[22] These abnormal cells may occur due to defects in the secretory apparatus but they can also occur in the case of mutated Ig-genes.[23]

Table 1. *Flow cytometric analysis of peripheral blood*

	Results	Reference		Results	Healthy age-matched controls
IgA (g/l)	<0.1	0.76-3.90	Total CD27+ (% of B cells)	23.6	27.7
IgM (g/l)	2.17	0.45-2.30	IgD+/CD27+ (% of B cells)	20.6	12.4
B cells (10^9/l)	0.37	0.10-0.30	IgD-/CD27+ (% of B cells)	3.0	15.3
T cells (10^9/l)	0.83	0.70-1.90	CD40 expression	N	N
Somatic hypermutation (%)	24.3	28-62%	CD40L expression	N	N

The analysis showed slightly increased absolute numbers of B cells, of which 23.6% had a memory phenotype (CD27+) and 3% were switched memory B cells (IgD-/CD27+).

Figure 1. *Mott cells in the May Grünwald-Giemsa stainings of the lymph node*

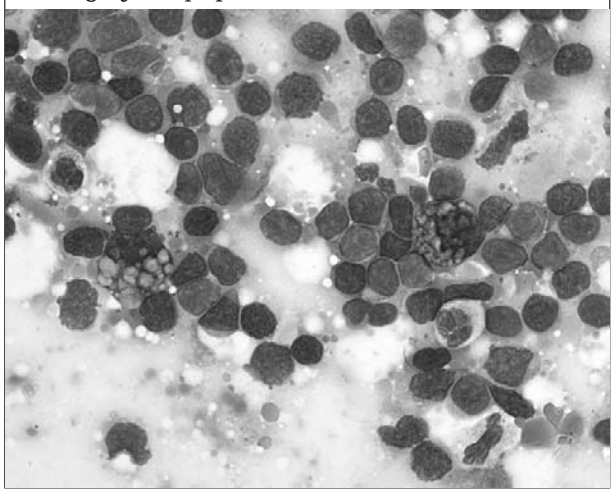
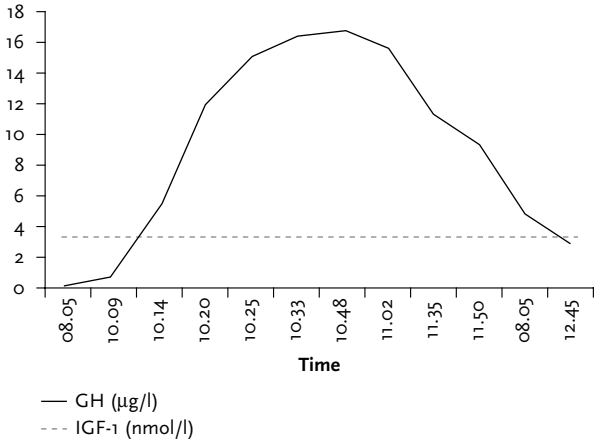


Figure 2. *Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) levels during the growth hormone-releasing hormone (GHRH) stimulation test*



Patient's GH response to GHRH infusion was normal. Starting point was well below the normal GH level (normal: 5 µg/l). In the course of a few hours (8.05 am to 12.45 pm) the GH level increased accordingly after GHRH stimulation and decreased again, to starting level. No IGF-1 response to GH elevation was seen. IGF-1 level remained low (normal: 15 nmol/l).

Functional assays

In February 2006 the growth hormone-releasing hormone (GHRH)-GH-IGF-1 axis was tested (*figure 2*) because of repeated low serum levels of GH and IGF-1 in a non-active disease condition (no infections). GHRH-GH function proved normal (from <1 ug/l to >17 ug/l) whereas GH-IGF-1 function showed to be impaired: circulating IGF-1 and BP-3 remained very low (3.4 nmol/l and 1 mg/l, respectively). Taken together, in this patient the arginine/GHRH stimulation test did not result in an increase in serum IGF-1, suggesting a defect in the IGF-1 synthesis/release.

IGF-1 levels of 14 other CVID patients were measured for comparison. Controls were aged between 30 and 75 years. All proved to have a higher IGF-1 level than the discussed patient, ranging from 8.8 to 29.0 nmol/l (*table 2*) (normal 13 to 35 nmol/l). When blood samples were taken, none of the patients were suffering from active disease (no infections).

Cortisol and adrenocorticotropic hormone (ACTH) were in the normal range, whereas testosterone was at the lower limit (9.8 nm/l); normal 10 to 30 nm/l). A subclinical hypothyroidism – thyroid stimulating hormone (TSH) level 7.6 mU/l (normal 11-25 mU/l) with fT4 level 15.6 pmol/l (normal 0.4 to 4.3 pmol/l) – was also detected.

Table 2. *IGF-1 serum levels of a small cohort of patients with hypogammaglobulinaemia*

Gender	Age	IGF-1 (nmol/l)	Gender	Age	IGF-1 (nmol/l)
♀	30	9.7	♀	49	12.7
♀	34	18.2	♀	76	15.0
♀	46	12.8	♀	35	29.1
♀	38	16.6	♂	53	14.0
♀	44	29.0	♂	70	10.6
♀	47	18.2	♂	43	18.7
♀	40	8.8	♂	44	22.1

Two of 14 showed a rather low level of IGF-1. Normal range is between 13 and 35 nmol/l with a negative relation to age.

Mutational analysis

STAT5B mutations have been linked to immune deficiency as well as GH and IGF-1 deficiency.[24-26] Moreover, the reduced proliferation in the cells from this patient was very similar to the reduced proliferation observed before in a

patient with an STAT5B mutation (De Paus; unpublished data). The patient's STAT5B gene was sequenced in mRNA isolated from whole blood. No variations were detected. Subsequently all exons of the GHR and IGF-1 genes were sequenced, including at least 20 nucleotides (nt) upstream and downstream of each exon, in genomic DNA. One known silent polymorphism, rs6179, was present heterozygously in GHR; no other variations were detected in either GHR and IGF1. The IGF-1 gene was amplified from mRNA isolated from whole blood. Upon analysing the four known IGF-1 splice variants IGF-IA, IGF-IB, IGF-IA' and IGF-IB',[27] amplification of the IGF-IB splice variant failed, whereas from RNA of six healthy control individuals the IGF-IB splice variant could be amplified. Intron 4 (1505 nt) and 600 nt of the promoter upstream of exon 1 were sequenced from genomic DNA, no aberrations were found.

DISCUSSION

The endocrine system has close interactions with the immune system. Hormones such as oestrogens have been shown to inhibit B cell differentiation significantly.[28,29] Hormones and receptors of the somatotropin axis are expressed during B cell development suggesting a role in B cell differentiation. Gene expression profiles of precursor B cell subsets of healthy donors show that IGF2-R and IGFBP-3 are expressed during B cell differentiation whereas IGF1-R is not upregulated (unpublished data). GH is also expressed and shows the highest expression during the pre-B-II cell stage suggesting that GH may be involved in the B lymphoid differentiation.

In this patient the B cell compartment shows a normal differentiation pattern ruling out that normal IGF-1/BP-3 levels are a major factor in the B cell differentiation; however, somatic hypermutation (SHM) was low. Germinal centre (GC) B cells that have acquired high affinity for the immunising antigen – through SHM – form long-lived plasma cells. Affinity maturation is driven by a tightly controlled mechanism that selects antibodies with the greatest possibility of neutralising foreign antigens. Because the body can sustain only limited numbers of plasma cells, this 'quality control' over plasma cell differentiation is likely critical for establishing effective humoral immunity.[30] The germinal centre CD38+ B-cell population and the mantle-zone CD39+ B-cell population display similar levels of hGHR expression, to our knowledge no reports have been published concerning IGF-1R expression in GCs. The aberrations in T cell function and proliferation observed, fit within the CVID profile; up to 30% of the patients with CVID have abnormal T cell function or numbers.

Functionally, Kimata and Yoshida investigated the effect of GH and IGF-1 on human plasma cell responses *in vitro* back in 1994. They found that both GH and IGF-1 enhance the production of IgG1 and IgE producing plasma cells.[15] Clinically, the combination of immunoglobulin and GH deficiency has been described previously in two case reports.[19,20] IGF-1 deficiency, hearing loss, growth retardation, and immune dysregulation have been attributed to STAT5B mutations; however, Walenkamp *et al*. recently described a patient with an STAT5B mutation and no clinical immune deficiency.[7,24]

Normal IGF-1 levels were found in most patients with immunoglobulin deficiency as shown in *table 2*. Moreover, one patient, who is being treated for hypogammaglobulinaemia, has a contradictory profile: thymic hyperplasia and CVID (possibly Good's syndrome) and elevated serum levels of GH and IGF-1. GH can be synthesised by the thymus.[31] Two dominant splice variants of IGF1 exist: IGF1-IA, consisting of exon 1,3,4 and 6 and IGF1-IB, consisting of exon 1,3,4 and 5. In this the IGF1-IB mRNA that contains exon 5 was absent. Part of exon 5 encodes for a growth-promoting peptide. In a study analysing IGF1 splice variants in GH-deficient patients it was found that the exon 5-containing splice variants were nearly absent while they could be strongly induced by treatment with GH.[32] The observed absence of the IGF1-IB splice variant in our patient may therefore be due to low levels of GH.

The abnormalities in the patient described are rare: CVID has a prevalence of 1:25,000 to 100,000 and primary IGF-1 deficiency (other than Laron's syndrome) is so rarely described that prevalence/incidence rates are currently unknown. The combination therefore makes a common defect in both systems, IgG and IGF-1 synthesis and release, more likely. To our knowledge immune deficiency is not a component of Laron's syndrome.

Several genetic syndromes have been described with some resemblance to this patient. Ataxia telangiectasia was excluded. Most clinical features of this patient were in agreement with Roifman's syndrome; however, skeletal and humoral and cellular parameters were different.

The fact that the B lymphocyte development in peripheral blood and plasma cells was found in the patient's lymph nodes suggests a normal B cell differentiation. However, the percentage of plasma cells in the bone marrow was extremely low, which may indicate a possible defect in the homing of plasma cells/plasmablasts back to the bone marrow. Bone marrow plasma cells produce approximately 80% of the serum IgG and thus are essential for humoral memory and stable antibody titres.

CONCLUSION

We therefore hypothesise that in this patient one of the two previously described mechanisms may be responsible for the low IgG levels; either IGF-1 deficiency results in IgG deficiency or both the immunoglobulin deficiency and primary IGF-1 deficiency are caused by a common defect in both pathways. The low incidence rate of both disorders, normal IGF-1 levels in the CVID group, normal immune competence in Laron's syndrome and lack of IGF-1R in human lymphocytes favour the last hypothesis.

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