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Frequent IgG subclass and mannose binding lectin deficiency in patients with chronic fatigue syndrome

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ABSTRACT

Chronic fatigue syndrome (CFS) is a severe disease characterized by various symptoms of immune dysfunction. CFS onset is typically with an infection and many patients suffer from frequently recurrent viral or bacterial infections. Immunoglobulin and mannose binding lectin (MBL) deficiency are frequent causes for increased susceptibility to infections. In this study we retrospectively analysed 300 patients with CFS for immunoglobulin and MBL levels, and B-cell subset frequencies. 25% of the CFS patients had decreased serum levels of at least one antibody class or subclass with IgG_3 and IgG_4 subclass deficiencies as most common phenotypes. However, we found elevated immunoglobulin levels with an excess of IgM and IgG_2 in particular in another 25% of patients. No major alteration in numbers of B cells and B-cell subsets was seen. Deficiency of MBL was found in 15% of the CFS patients in contrast to 6% in a historical control group. In a 2nd cohort of 168 patients similar frequencies of IgG subclass and MBL deficiency were found. Thus, humoral immune defects are frequent in CFS patients and are associated with infections of the respiratory tract.

1. Introduction

Chronic fatigue syndrome (CFS), also known as myalgic encephalomyelitis (ME), has been classified as a complex neuroimmunological disorder under ICD 10 G93.3 by the World Health Organization (WHO) [1]. CFS is defined by sustained physical and mental exhaustion accompanied by various symptoms including headache, sore throat, myalgia and arthralgia, lymphadenopathy and neurocognitive impairments [2]. CFS onset is frequently triggered by an acute infection and a subset of patients suffers from frequently recurring mostly respiratory tract infections [3]. Interactions between chronically activated inflammatory pathways, impaired immune function and resultant enhanced production of oxidative and nitrosative stress, and mitochondrial dysfunction are considered as putative key pathological mechanism [4].

Immunological alterations described in CFS include changes in cytokine profiles [5,6], decrease of natural killer (NK)-cell function [7–9], elevated levels of complement activation products [10], autoantibodies [11,12] and circulating immune complexes [11]. Authors found both increased expression of T-cell activation markers and unchanged or lower expression in CFS patients [13,14].

B cells seem to play an important role in the immunopathology of CFS since treatment using the anti-CD20 antibody Rituximab demonstrated clinical effectiveness [15]. One study hypothesized that CFS patients have a B-cell immunodeficiency as a decrease of $CD19/IgM^+$ B cells was detected [16], while two other studies revealed elevated levels of $CD19^+$, $CD20^+$ and $CD21^+$ B cells [7,17]. A recent study reported expanded transitional and naïve B

Abbreviations: CFS, chronic fatigue syndrome; Ig, immunoglobulin; IGHC, immunoglobulin heavy constant region; IVIG, intravenous immunoglobulin therapy; MBL, mannose binding lectin; ME, myalgic encephalomyelitis; NK cell, natural killer cell; WHO, World Health Organization (WHO); U, units.

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cells and reduced plasmablasts [18]. Further, alterations in serum immunoglobulin levels have been described in CFS. One study noted decreased immunoglobulin A (IgA) and immunoglobulin E (IgE) levels in 21 CFS patients [19]. Bates et al. found increased immunoglobulin G (IgG) [11], while diminished serum concentrations were measured by the group of Wakefield et al. They found evidence of IgG_1, IgG_2 and IgG_3 deficiencies in a study of 78 CFS patients [20]. In contrast, the case–control study by Bennett et al. observed only elevated IgG_1 serum concentrations within the CFS group [21]. In a recent study from our group a deficient EBV-specific B-and T-cell response was found in the majority of patients with CFS [22]. Clinical trials of immunoglobulin replacement therapy have inconsistent results. A randomized, placebo-controlled, double-blind study by Lloyd et al. showed that CFS symptoms improved by immunoglobulin substitution in the majority of patients [23]. However, in a second randomized study by Peterson et al. patients had no benefit from immunoglobulin infusions [24].

Next to immunoglobulins mannose-binding lectin, MBL, plays an important role in the antibacterial defence. MBL synthesized in the liver binds to mannose and specific carbohydrates on microbes and is capable of eliminating pathogens directly by opsonophagocytosis and indirectly by complement activation (lectin pathway) [25,26]. MBL deficiency is found at a frequency of 4% in the Caucasian population [27–31]. MBL levels are genetically determined by polymorphism in the *mbl2* gene promotor region with allele A for the normal allele, and allele B (GGC to GAC), C (GGA to GAA), and D (CGT to TGT) for MBL amino acid substitutions. Any combination of allele A with the variants B-D are designated as A/O which results in low to undetectable levels of MBL [27]. In a previous study, we could show that in adults low MBL levels are associated with an increased susceptibility to respiratory and urogenital infections [28]. Other studies revealed an association with more severe outcome of infections [29–31]. To our knowledge there is no study available analysing MBL deficiency in CFS patients yet.

In the present study we analyzed two large cohorts of CFS patients for immunoglobulin and MBL levels and the association with infections. Deficiency of both IgG subclasses and MBL was found more frequently in CFS patients and was associated with increased susceptibility to infections.

2. Materials and methods

2.1. Patient collective, inclusion and exclusion criteria

We retrospectively analyzed 300 CFS patients who had been referred to our immunodeficiency outpatient clinic between 2011 and 2012. A 2nd cohort of 168 patients who were referred in 2013 was prospectively analyzed.

CFS was diagnosed based on Fukuda criteria [2] and exclusion of other medical or neurological diseases. In the 2nd cohort, disease symptoms fulfilled Canadian criteria as well [32]. Enhanced susceptibility to infection was defined as having a history of four or more respiratory tract infections per year and/or a history of severe infections (e.g. pneumonia). Infection history was assessed retrospectively from patients files in the first cohort and prospectively by the physician and documented in a separate file in the second cohort. Frequent reactivation of herpetic lesions was defined as four or more per year. Patients with chronic systemic steroid or immunosuppressant therapy or a diagnosis of primary immunodeficiency were excluded from this study. The study was approved by the Ethics Committee of Charité Universitätsmedizin Berlin in accordance with the 1964 Declaration of Helsinki and its later amendments. All persons gave informed consent prior to their inclusion in this study.

2.2. Data collection

With the aid of medical reports and the Charité Medical Information System (SAP), we created a database program with Microsoft Access 2007 and Visual Basic for Access (VBA) which incorporated the following information: age; sex; results of the determination of immunoglobulin classes and IgG subclasses; MBL serum concentration; B-cell subset distribution.

2.3. Quantification of MBL and immunoglobulin classes

MBL levels were analyzed by the Charité's interdisciplinary immunological laboratory (Labor Berlin GmbH). Reference and cut-off values for MBL base on our analysis of a large healthy control group in a previous study [28]. Values $\leqslant$100 ng/ml are defined as MBL deficiency. MBL levels were measured by ELISA (AntibodyShop, Gentofte, Denmark). Serum immunoglobulins IgA, IgM and IgG were determined at Labor Berlin GmbH by immunological turbidity test (Roche Diagnostics). IgG subclasses were measured by immunoturbidimetric technique (The Binding Site). Reference values refer to Schauer et al. [33].

2.4. Flow cytometric analysis of B-cell subsets

EDTA blood was used for determination of B cells. Reference values of B cell subsets analyzed in Labor Berlin GmbH refer to Warnatz and Schlesier [34]. The cut-offs were defined by the 5 to 95 percentile. Cells were stained with CD19, $CD27^{-}IgD^{+}IgM^{+}$ for naïve B cells, $CD27^{+}IgD^{+}IgM^{+}$ for marginal zone B cells, $CD27^{+}IgD^{-}IgM^{-}$ for class-switched memory B cells, $CD21^{int}CD38^{hi}IgM^{hi}$ for transitional B cells, and $CD21^{int}CD38^{hi}IgM^{-(+)}$ for plasmablasts, respectively. The cells were analyzed by flow cytometry in our immune diagnostic laboratory at Labor Berlin GmbH. In a cohort of 20 patients and 20 healthy controls frequencies of B cells subsets were comparatively determined in our research laboratory (antibodies from Biolegend).

2.5. Statistical analysis

Statistical analysis was performed using Graph Pad Prism version 5.0. All data were distributed non-parametrically and analyzed using the Kolmogorov–Smirnov Normality Test. Mann–Whitney *U* test and Chi square test were used and results were statistically significant at $p < 0.05$.

3. Results

3.1. Characteristics of study population

We retrospectively analyzed 300 patients with CFS who presented at our outpatient clinic (Table 1). Diagnosis was based on exclusion of other medical conditions and Fukuda criteria with patients fulfilling on average six of eight minor criteria. 89% of patients indicated post-exertional malaise as one of the most characteristic criterion of this disease. The performance status according to Bell's disability rating was median 30, corresponding to moderate to severe symptoms at rest and a reduced overall activity level (Fig. 1) [35]. A history of frequent recurrent respiratory tract infections (RRTI) was reported in 33% of patients including 7 patients with pneumonia and 8% of patients reported frequently recurrent herpes simplex lesions. Consecutively, a second cohort of 168 patients was analyzed who all fulfilled the Canadian criteria. In these patients the infection history was prospectively assessed and 35.7% of patients suffered from frequent RRTI. Details are shown in Table 2.