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Cellular and humoral influenza-specific immune response upon vaccination in patients with common variable immunodeficiency and unclassified antibody deficiency

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ABSTRACT

Background: Immunization against seasonal influenza with inactivated vaccine is recommended for patients with common variable immunodeficiency (CVID). However, humoral vaccine response in CVID patients is frequently impaired and current knowledge on T cell vaccine response in CVID and other patients with antibody deficiency is poor.
Objective: In the present study, we comparatively analyzed the antibody and T cellular immune response of patients with CVID and unclassified antibody deficiency to influenza vaccination in the season 2013/2014.
Methods: Eight patients with CVID, 8 patients with unclassified antibody deficiency and 9 healthy controls were vaccinated with a single dose of non-adjuvanted seasonal influenza vaccine. Before and 3 weeks after the vaccination antibody titers against the strains A/California/7/2009, A/Texas/50/2012, and B/Massachusetts/02/2012 included in the vaccine were measured by hemagglutination inhibition testing. Additionally, vaccine-specific T cell cytokine response was determined by stimulation with the complete vaccine *in vitro*.
Results: Whereas all healthy controls responded to vaccination with serum antibody titers, only 1/8 CVID patients and 4/8 patients with unclassified antibody deficiency showed a response against at least 1 of the 3 vaccine strains. However, 7/8 of the CVID and 6/8 of the patients with unclassified antibody deficiency had similar frequencies of vaccine-induced IFN-γ, TNF-α and IL-2 producing $CD40L^+$ T cells as the control group.
Conclusion: Our data suggest that most CVID and unclassified antibody deficiency patients benefit from seasonal influenza vaccination by mounting a cellular response.

1. Introduction

Common variable immunodeficiency (CVID) is the most commonly diagnosed clinically relevant primary immunodeficiency disorder (PID) with an estimated prevalence of 1:25,000–50,000 [1,2]. According to the current criteria of the European Society for Immunodeficiencies (ESID), CVID is defined by diminished immunoglobulin IgG and IgA with or without reduced IgM levels as well as reduced frequencies of switched memory B cells and/or diminished vaccine antibody responses [http://esid.org/Working-Parties/Registry/Diagnosis-criteria]. Patients with combined hypogammaglobulinemia (reduced IgG and IgA and/or IgM) but normal

Abbreviations: CVID, common variable immunodeficiency; ESID, European Society for Immunodeficiencies; HI, hemagglutination inhibition; PBMCs, peripheral blood mononuclear cells; PID, primary immunodeficiency disorder; RRTI, recurrent respiratory tract infections; uAD, unclassified antibody deficiency.

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levels of switched memory B cells and normal or impaired vaccine response, otherwise not fulfilling ESID CVID criteria are categorized as unclassified antibody deficiency. In a recent study by Driessen et al. comparing CVID patients with 21 patients with unclassified antibody deficiency it was shown, that both groups suffer to a similar extent from infections, while non-infectious complications such as autoimmunity, hepatosplenomegaly or granulomatous disease were only found in CVID patients [3].

Standard treatment for CVID is immunoglobulin replacement therapy and antibiotics. As in CVID patients, treatment decision on immunoglobulin replacement in unclassified antibody deficiency is depending on the severity and frequency of infections. Vaccination is recommended, despite a frequently impaired humoral response in PID patients. Data about T cell responses is sparse and contradictory [4–6], but previous studies in patients with X-linked agammaglobulinemia and in patients with Hyper IgM syndrome (HIGM1) have shown T cell responses against vaccination antigens [7–10].

Influenza can lead to severe respiratory tract infection in PID patients. Due to its high mutation rate, immunoglobulin replacement therapy can only partially protect from the seasonal influenza. Annual vaccination with the "yearly adapted vaccine" is an effective means of prevention and control of influenza in immunocompetent individuals and is also recommended for patients with PID, even in those with a known poor antibody response. In addition to the development of protective antibodies after vaccination, the induction of cell mediated immunity is considered to be of critical importance [11].

To our knowledge so far only one study from the Netherlands including 15 patients and three case reports from Norway addressed the issue of influenza vaccine responsiveness in CVID patients [12,13]. Van Assen et al. showed in their cohort of 15 CVID patients neither an increase of A/H1N1 or A/H3N2-specific antibodies HI assay nor IFN-γ-producing $CD4^+$ T cells by flow cytometry in comparison to healthy controls [12]. In contrast, the report from Norway found a response to the pandemic A/California/7/2009 (H1N1)-like split virus (X179a) influenza vaccination in 2 of 3 patients with CVID. This response was determined by an increase in hemagglutination-inhibiting antibodies and vaccine-specific IFN-γ, TNF-α and IL-2-producing $CD4^+$ T cells determined by flow cytometry [13].

In our study, we evaluated the response to influenza vaccination in patients with CVID and unclassified antibody deficiency. We could show that while the humoral immune response was strongly impaired, a T cell response against the vaccine was detected in most patients.

2. Material and methods

2.1. Human blood samples and vaccination

Patients with confirmed diagnosis of CVID according to ESID criteria, and patients with unclassified antibody deficiency were recruited from the outpatient clinic for immunodeficiencies at the Institute of Medical Immunology at the Charité Universitätsmedizin Berlin between 2013 and 2014. 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 patients and healthy controls recruited from staff gave written informed consent.

Heparin blood was taken prior to and 21 days after the intra-muscular vaccination against influenza using 0.5 ml of the non-adjuvanted trivalent vaccine VAXIGRIP® 2013/2014 (lot number: K0296-1) from Sanofi containing the inactivated strains A/California/2009 (H1N1, NYMC X-179 A), A/Victoria/361/2011 (H3N2, NYMC X-223A derived from A/Texas/50/2012), and B/Massachusetts/02/2012, all at 15 μg, respectively.

Response to pneumococcal vaccination was previously determined 4–6 weeks after polysaccharide vaccine administration (Pneumovax23®) at Labor Berlin GmbH using an ELISA for anti-pneumococcal IgG and IgG_2 (Binding Site) as part of the routine diagnostics.

2.2. Quantification of immunoglobulins and cell subsets

Serum IgG, IgA and IgM were determined at Labor Berlin GmbH by immunological turbidity test (Roche Diagnostics). CD4 and CD19 counts and frequencies of CD19+ B cell subsets according to the EURO class panel were determined by flow cytometry at Labor Berlin GmbH [14].

2.3. Hemagglutination inhibition assay

Influenza-specific serum antibody titers were measured by standard hemagglutination inhibition (HI) assay, using the strains A/California/7/2009 (H1N1pdm09), A/Texas/50/2012 (H3N2), and B/Massachusetts/02/2012 and erythrocytes from turkey hens as previously described [15].

2.4. Flow cytometric analysis of T cell cytokines

Peripheral blood mononuclear cells (PBMCs) were isolated from heparin blood samples by density gradient centrifugation and frozen at −80 °C. Thawed PBMCs were either left unstimulated or stimulated for 20 h at 37 °C with 3 μg/ml superantigen SEB (Sigma–Aldrich) as positive control or with 3 μg/ml of VAXIGRIP®, respectively. After the first 2 h of stimulation, brefeldin A (Sigma–Aldrich) was added as secretion inhibitor. Cells were stained extracellularly with CD3 PercpCy5.5 (Biolegend), CD4 APC H7 (Biolegend), life-dead Pacific Orange (Life Technologies), CD40L PE (Becton, Dickinson and Company) and lyzed and permeabilized. Afterwards intracellular staining was performed with IFN-γ FITC (Becton, Dickinson and Company), TNF-α PECy7 (eBioscience) and IL-2 APC (Becton, Dickinson and Company), respectively. Cells were measured at a LSRII (Becton, Dickinson and Company) and analyzed with FlowJo software.

2.5. Statistical analysis

Statistical data analyses were done using the software SPSS Statistics 19. Nonparametric statistical methods were used. Continuous variables were expressed as median and interquartile range (IQR) and geometric mean with 95% CI for fold induction in Fig. 3. Univariate comparisons of two independent groups were done using the Mann–Whitney-*U* test or Fisher's exact test, of dependent groups using Wilcoxon signed-rank test, respectively. A two-tailed *p*-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

Influenza-specific antibody and T cell responses were analyzed in 9 healthy volunteers, 8 patients with CVID and 8 patients with unclassified antibody deficiency (uAD) diagnosed according to ESID criteria. The patient characteristics are shown in Table 1. All patients suffered from recurrent respiratory tract infections (RRTI) and all but one patient (uAD#1) received immunoglobulin replacement therapy with positive clinical response regarding infectious complications. 4 CVID patients and 1 patient with unclassified antibody deficiency had autoimmune diseases. All patients

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