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ASTUTE CLINICIAN REPORT

Late-Onset Disseminated *Mycobacterium avium intracellulare* Complex Infection (MAC), Cerebral Toxoplasmosis and Salmonella Sepsis in a German Caucasian Patient with Unusual Anti-Interferon-Gamma IgG_1 Autoantibodies

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Abstract
Purpose Since we described for the first time a patient with IgG_4 autoantibodies to IFN-γ more than 10 years ago, many patients with IFN-γ IgG_4 autoantibodies have been described, mostly in Mongolian/ Asian patients with a particular HLA background and in association with disseminated nontuberculous mycobacterial infections. Very recently, the first Caucasian US patient was reported and we now present the case of a 65-year old Caucasian woman with severe disseminated *Mycobacterium avium* infection, cerebral toxoplasmosis and *salmonella* sepsis who was tested positive for IFN-γ deficiency due to unusual anti-IFN-γ IgG_1 autoantibodies.
Methods IFN-γ production after ex vivo ConA stimulation of the patient's whole blood and isolated peripheral blood mononuclear cells was assessed. Anti-human IFN-γ antibodies were measured by Ig/Ig-subclass-specific ELISA. In vitro physiologic relevance and blocking capacity of IFN-γ-stimulation by patient's serum was analysed by flow cytometric assessment of cytokine-induced phosphorylation of $pSTAT1^{Y701}$.
Results Severely impaired IFN-γ production in the patient's whole blood but normal production in peripheral blood mononuclear cells in the absence of autologous serum was observed. High titre anti-IFN-γ antibodies of the IgG_1 subclass could be demonstrated in the patient's serum by ELISA. Further, the addition of patient's serum to IFN-γ-stimulated immune cells showed inhibition of STAT1 phosphorylation.
Conclusions IFN-γ autoantibodies of any IgG-isotype should be considered in patients with severe opportunistic infections independent of age at onset and ethnicity.

Keywords anticytokine antibody · autoantibody · interferon gamma (IFN-γ) · IgG_1 · non-tuberculous mycobacterium · MAC · NTM · MSMD · toxoplasmosis · secondary autoimmune-mediated immunodeficiency

Introduction

The first patient with anti-IFN-gamma (IFN-γ) autoantibodies suffering from severe *Mycobacterium cheloneae* and *Burkholderia cocovenenans* infection was reported in 2004 in a female patient from Thailand by our group [1]. Subsequently a second case report on IFN-γ antibodies from an Asian patient suffering from nontuberculous *mycobacteria* infection (NMI) and *Bacillus Calmette-Guérin* was published by British clinicians [2]. In 2005 Kampmann et al. described 3 cases with one South African patient and 2 UK resident patients with NMI and anti-IFN-γ autoantibodies [3]. Pastel et al. also screened 35 patients with either disseminated or pulmonary NMI of whom six East Asian descent patients

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had high-titer-neutralizing anti-IFN-γ IgG [4]. Further several Japanese patients with anti-IFN-γ autoantibodies were reported [5–7]. A study in 2012 analysing 97 patients from Thailand and Taiwan with disseminated NMI and other opportunistic infections described anti-IFN-γ autoantibodies in the majority of patients suggesting the presence of anti-IFN-γ autoantibodies to be frequent in Asia [8]. However, antibodies in those 16 patients, investigated for IFN-γ-specific subclasses were mostly of IgG_4 subclass. Recently O'Connell described the first US Caucasian patient with IFN-γ IgG_1-antibodies suffering from disseminated MAC [9].

Case

We report the case of a 65-year old, Caucasian woman from a rural German region, who presented in 2013 with acute hypercalcemia and weight loss at the Department of Infectious diseases, where a disseminated *Mycobacterium avium* infection of the lung, pancreas and multiple lymph nodes was diagnosed. Diagnosis was confirmed by acid-fast staining, PCR and culture in all three tissues. Histopathological analysis revealed epitheloid granuloma formation with massive neutrophil invasion. Despite triple tuberculostatic treatment for more than 4 months with rifampicin, myambutol and clarithromycin the patient developed a dramatic increased lymphadenopathy (Fig. 1) which prompted further immunological examination. The previous medical history revealed a cerebral, cerebellar and spinal toxoplasmosis with findings of bradycoites in a cerebellar biopsy 2 years earlier, which had left no neurological residuals after being treated with sulfadiazine/ pyrimethamine for 21 months. Furthermore, the patient had suffered from a severe infection with *Salmonella enterica* leading to sepsis and multi-organ failure 4 years earlier. The medical history before the age of 61 years was unremarkable and the patient did not suffer from any type of autoimmunity.

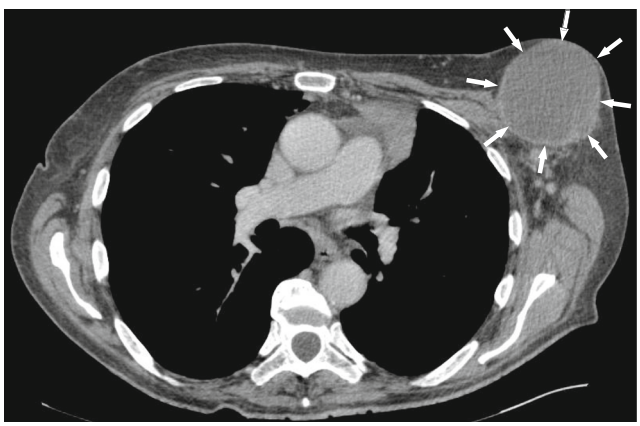

Fig. 1 MRI/CT of lymphadenopathy of a mycobacterial infected enlarging axillary lymph node under antibiotic treatment

Material and Methods

Clinical Samples

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll Hypaque from heparinized blood samples from the patient and healthy subjects. The study was approved by the Ethics Committee of Charité Universitätsmedizin Berlin (EA4/061/08). The patient and healthy controls gave informed consent.

Determination of Cytokines

Cytokines were determined by Cytometric Bead Array (CBA) (Human Th1/Th2 Cytokine Kit II, BD Biosciences) measured on a FACS Navios (Beckman Coulter) after stimulation of heparinized whole blood or PBMCs with ConcanavalinA (ConA) (Sigma) at 50 ng/ml in low endotoxin RPMI medium. Supernatants were taken after 24 h.

Inhibition of IFN-γ-induced STAT1 Phosphorylation

PBMCs of a healthy control donor were stimulated with 0.1, 1, 10, 100, and 1000 ng/ml of recombinant IFN-γ (Imukin, Boehringer Ingelheim). The cytokines for stimulation were preincubated in the healthy control/ patient's serum at a 1:20 dilution for 1 h and PBMC's were then stimulated with the IFN-γ/ serum mixture for 10 min. Similarly PBMC's were stimulated for 10 min with 10 ng/ml IFN-γ at different serum concentrations ranging from 1:312 to 1:20.000. After 8 min of stimulation a mixture of surface antibodies for CD3, CD16, CD56, CD19 and CD14 was added (Beckman Coulter). Stimulation was stopped after 2 additional minutes by addition of 5 μl paraformaldehyde for another 10 min at 37 °C according to the manufacturers' instructions (PerFix Expose staining kit, Beckman Coulter) for fixation and permeabilization. Cells were then simultaneously stained with AF488- labelled primary antibodies against pSTAT1 (pY701) (clone 58D6, Cell Signaling Technology) and CD45 (Beckman Coulter) for 30 min at RT in the dark. Flow cytometric measurement was performed on a Navios (Beckman Coulter) and Navios-software 1.2., FlowJo 9.2., and Graph Pad Prism 5 software were used for data analysis.

Determination of Anti-IFN-γ Autoantibodies

A 96-well flat-bottom MaxiSorp® plate (Nunc) was coated overnight with 100 μl/well IFN-γ (Imukin, Boehringer Ingelheim) at 1 μg/ml in sodium-carbonate buffer at pH 9.6 and blocked with PBS/ 5 % BSA for 2 h at 37 °C. Serum samples were incubated for 2 h at room temperature and were detected with HRP-labelled goat-anti-human IgG (Star106P, AbD Serotec, RRID AB_323001, 1:16000) or gout-anti-

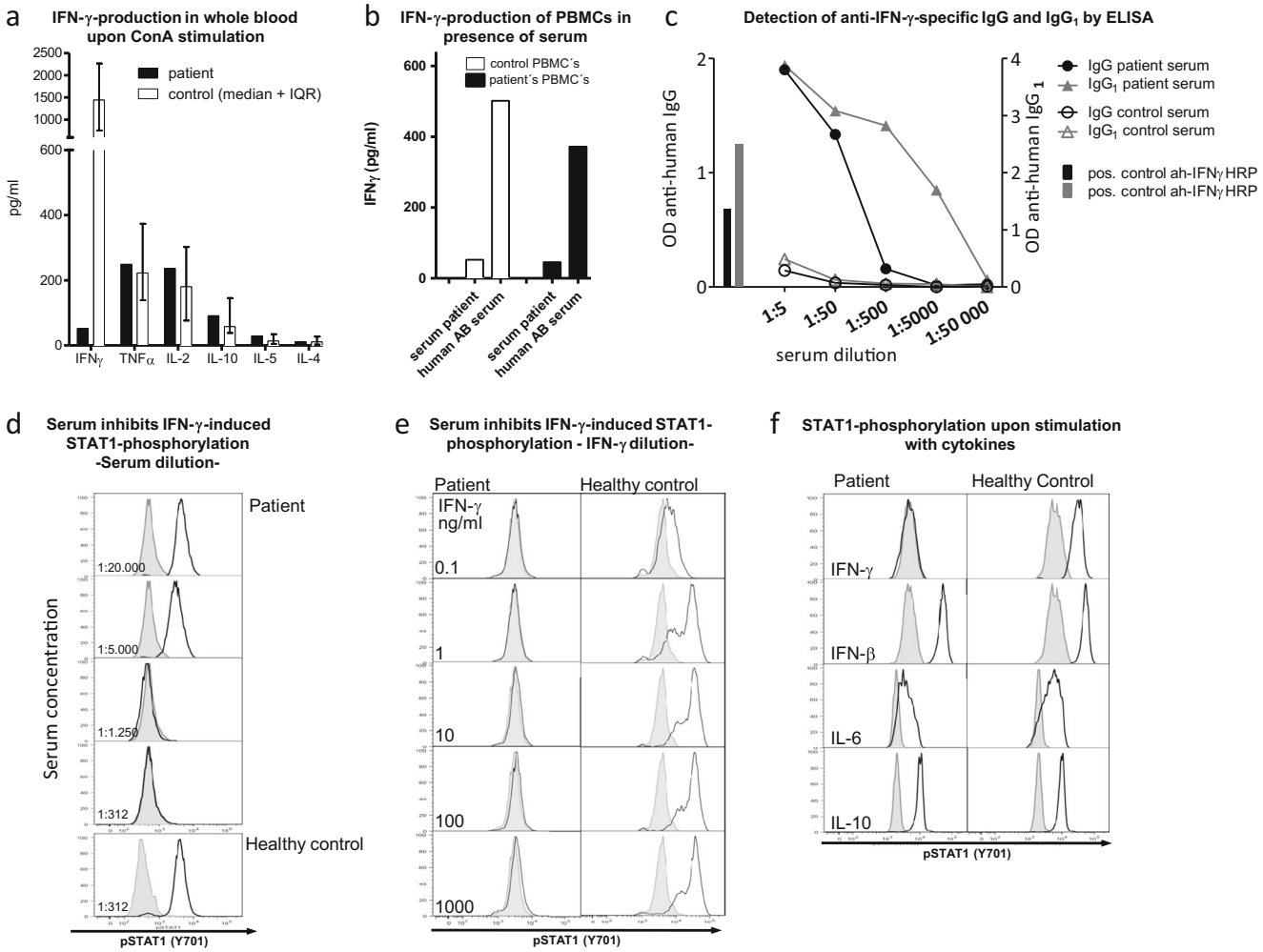

Fig. 2 Detection of functional anti-IFN-γ autoantibodies. **a** CBA for depicted cytokines from ConA-stimulated whole blood supernatants revealed absent IFN-γ in the patient (*black*) and normal levels in the control group of 25 healthy age-and sex-matched donors (*white*, Median ± IQR). **b** IFN-γ in the supernatant of ex vivo ConA-stimulated (20 μg/ml) PBMCs of the patient (*black*) and control (*white*) co-incubated with 20 % AB serum of healthy controls or patient serum, respectively. **c** Anti-IFN-γ specific IgG and anti-IFN-γ specific IgG_1 were detected in patient serum by ELISA at depicted dilutions. As positive control for the ELISA a HRP-labelled goat-anti-human-IFN-γ antibody was used. **d** STAT1 phosphorylation at Y701 in $CD14^+$ monocytes in presence of depicted dilutions of serum from the patient or a healthy control either unstimulated (*grey filled*) or after stimulation with 10 ng/ml of recombinant IFN-γ (*not filled*). **e** STAT1 phosphorylation at Y701 in monocytes in presence of 20 % serum of the patient or a healthy control either unstimulated (*grey filled*) or after stimulation with indicated concentrations of recombinant IFN-γ (*not filled*). **f** STAT1 phosphorylation is shown unstimulated (*grey filled*) or after stimulation (*not filled*) with 10 ng/ml of IFN-γ, IFN-β, IL-6 or IL-10 in the presence of patient's or control serum

human IgG_1 (clone MH161-1, Fitzgerald, 1:4000, RRID AB_1286031) and developed by the addition of stabilized TMB (from Kit 2142, Celestis) and measured at 450/540 nm at an VersaMax reader (Molecular Devices). As positive control for the IgG and IgG_1 ELISA goat-anti-human-IFN-γ-IgG-HRP (from Kit 2142, Celestis) was used at 1:100 dilution.

Results

The patient's immune status revealed normal $CD4^+$ T cells and other lymphocyte subpopulations, granulocytes and $CD14^+$ monocytes. Levels of immunoglobulins were normal or enhanced for total IgG (18.9 g/l) and IgG_1 (14.4 g/l). HIV-testing was negative.

IFN-γ was barely detectable upon 24 h ConA ex vivo stimulation (58 pg/ml, expected range from 100 healthy subjects between 286 and 2911 pg/ml) in the supernatant of the patient's cells, whereas the level of other cytokines was normal (Fig. 2a). Normal IFN-γ levels were measured when patients PBMC were stimulated in the presence of serum of healthy controls (Fig. 2b). High titres of anti-IFN-γ IgG antibodies of IgG_1 subclass could be shown by ELISA (Fig. 2c), whereas specific antibodies against IFN-γ of the subclasses IgG_2, IgG_3 and IgG_4 were not found (data not shown). To assess physiologic relevance of the anti-IFN-γ antibodies, IFN-γ-induced

phosphorylation of $pSTAT1^{Y701}$ in PBMCs was analysed by flow cytometry in presence of different serum concentrations. Indeed, $pSTAT1^{Y701}$ was completely abolished in monocytes preincubated with the patient's serum up to 1:1.250 dilution and in the presence of up to 1000 ng/ml IFN-γ at 20 % serum concentration (Fig. 2d/e). At a 1:20.000 dilution STAT1 phosphorylation was no longer inhibited. Further, no inhibition was observed upon stimulation with IFN-β, IL-6, and IL-10, respectively (Fig. 2f). Follow up over almost 1 ½years during therapy showed until now almost unchanged anti-IFN-γ titres and in vitro physiologic relevance by IFN-γ signal inhibition (data not shown).

Discussion

The 2014 updated IUIS classification added as ninth category of primary immunodeficiencies (PIDs) the so called "Phenocopies of PIDs" including anticytokine autoantibodies [10]. To date the majority of anticytokine antibodies described are anti-IFN-γ. The vast majority of patients with anti-IFN-γ antibodies were found to be of Asian origin. So far only one Caucasian patient has been recently described [9]. In a study from the UK, the ethnicity of the two patients described is not mentioned [3].

The most common opportunistic infectious pathogen described in combination with anti-IFN-γ autoantibodies is *Mycobacterium avium*. Associated infections with group D *Salmonella* and *Varicella zoster* were reported [3, 8, 11, 12]. Salmonella infections due to IFN-γ-autoantibodies have been found in two other patients until now only [13, 14]. In the currently biggest study on patients with IFN-γ autoantibodies, IgG-subclasses were determined in a subset of patients revealing anti-IFN-γ IgG_4 levels, and low levels of anti-IFN-γ IgG_3 in 8 of 9 patients. Of those, 4 patients had anti-IFN-γ IgM in addition [8]. Recently, the first Caucasian US patient with disseminated MAI and anti-IFN-γ IgG_1 antibodies was reported [9]. Our Caucasian woman is the second patient described with an acquired IgG_1 anti-IFN-γ autoantibody. While IgG_4 antibodies are not able to bind C1q, IFN-γ -IgG_1 complexes may mediate complement activation via C1q binding.

Chi et al. reported an association of anti-IFN-γ autoantibodies with HLADQB1*05:02 (odds ratio of 7.16) and HLA-DBR1*16:02 (odds ratio of 8.68) in 17 patients analysed [15]. The prevalence of HLADQB1*05:02 and HLADRB1*16:02 is 5 and 35-times, respectively, higher in Taiwan compared to a German population [16, 17]. This HLA distribution may be one explanation for the high proportion of Asian IFN-γ autoantibody patients described so far. Our patient had a HLA-DRB1*03:01 and HLA-DQB1*02:01 phenotype. Interestingly, these alleles were also described in one of the 17 analyzed Taiwan patients.

After the initial deterioration the patient showed a good clinical and radiomorphological response under continued antituberculostatic treatment. However, after 18 months of therapy the patient developed an acute spondylodiscitis due to a biopsy-proven MAC infection. Antibiotic treatment often fails to control mycobacterial infection in patients with IFN-γ autoantibodies. Efficacy of immunomodulatory therapy is described in some patients including high dose IFN-γ therapy [2, 3] and rituximab [18, 19], which is initiated in our patient as well.

Conclusions

In our patient high titer functional IFN-γ autoantibodies could be demonstrated resulting in severe immunodeficiency with life-threatening MAI, toxoplasmosis and *salmonella* infection. IFN-γ autoantibodies as a cause of IFN-γ deficiency should be considered in patients with severe opportunistic infections independent of age at onset and ethnicity.

Conflict of Interest The authors declare that they have no conflict of interest.

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