Brief report

Naturally occurring anti-IFNγ auto-antibody and severe infections with Mycobacterium cheloneae and Burkholderia cocovenenans

Anti-IFNγ auto-antibody with highly neutralizing activity

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Abstract

Recently various genetic defects in IFN\(_\gamma\) mediated immunity have been described including mutations in the interferon-\(\gamma\) receptor 1 (IFNgR1) and receptor 2 (IFNgR2), Stat1 and IL-12 receptor beta 1 (IL-12Rb1) and IL-12 p40 genes. These mutations are associated with the occurrence of severe infections with intracellular pathogens especially nontuberculous mycobacteria and vaccine-associated BCG. Here we report on a previously healthy adult patient primarily presenting with severe infections with Burkholderia cocovenenans and subsequently Mycobacterium cheloneae. We found a strong inhibitory anti-IFN\(_\gamma\) activity in the patient’s plasma and identified a high-affinity neutralizing anti-IFN\(_\gamma\) autoantibody. Unfortunately, the patient died due to severe sepsis before knowing the nature of the inhibitory activity. The application of alternative therapeutic approaches such as IVIG or immunoadsorption may have been beneficial in this case. Screening for neutralizing anti-IFN\(_\gamma\) autoantibodies should supplement testing for IFN-\(\gamma\) and IL-12 pathway defects in patients with recurrent infections with intracellular pathogens, especially with NTM. conny.hoeflich@charite.de.

Introduction

IFN\(_\gamma\) is synthesized by activated T and NK cells and strongly stimulates monocytes / macrophages increasing their microbicidal activity, antigen presentation function and production of pro-inflammatory cytokines upon contact with microbial stimuli. Recently, genetic defects in the IFN\(_\gamma\) receptor system have been described in patients with vaccine-associated BCG or nontuberculous mycobacterial (NTM) infections demonstrating the importance of IFN\(_\gamma\) mediated immunity in human host defense against intracellular pathogens (1-3). In addition, IFN\(_\gamma\) has been shown to be an obligatory host survival factor in a murine model of Burkholderia pseudomallei infection (4). Therapeutic consequences for patients with IFN\(_\gamma\) receptor defects depend on the kind of defects: patients with partial defect may do well with intensified antibiotic treatment and administration of IFN\(_\gamma\), whereas patients with complete IFN\(_\gamma\) receptor deficiency may only profit from bone marrow transplantation (5,6). Beside IFN\(_\gamma\) receptor defects, abnormalities in STAT-1, IL-12 receptor beta 1 and
IL-12 p40 expression have been reported in some patients with NTM infections supplementing the variety of causes for impaired IFNγ mediated immunity (7,8). Here we report on a patient with an acquired defect of IFNγ mediated immunity presenting with severe infections with Burkholderia cocovenenans and NTM.

**Materials and methods**

*Case report.* A 25 year old female from Thailand was admitted to the hospital due to necrotizing lymphadenitis, pneumonia, and tonsillitis. Three months earlier, she had developed severe community acquired pneumonia with negative bacteriological results in another hospital. She had been living in Germany for the last 8 years and had last been in Thailand 4 years ago. Her past medical history as well as the family history were unremarkable as far as known (father unknown). Diagnostic lymphadenectomy revealed an accumulation of neutrophil granulocytes without signs for malignancy. Microorganisms were not detectable. Following tonsillectomy she developed bilateral pneumonia, splenic and intracerebral abscesses and osteomyelitis. By this time *Burkholderia* spp. was isolated from lymphadenoid tissue, different wound lesions, bronchial secretion and from a fluid containing pustule at the left thigh. By comparative 16S rDNA sequence analysis the isolate was classified as *Burkholderia cocovenenans* (syn.: *B. gladioli*). Additional colonizing microorganisms, *Candida* spp. and *Enterococcus faecalis*, were isolated from bronchial secretions. Mycobacteria were not detected and HIV serology was negative. *Burkholderia cocovenenans* is a plant pathogen and invasive infections in humans have been described in patients with chronic granulomatous disease and in patients with cystic fibrosis upon starting immunosuppressive therapy after lung transplantation (9,10). Analysis of phagocytic function and respiratory burst activity of the patient’s peripheral blood phagocytes was normal as was LPS-induced monocyte TNFα production. The flow cytometric analysis of immune cells revealed abnormalities compatible with systemic inflammation and reaction against intracellular antigens. Extensive examinations for any autoimmune disorder revealed negative results. After the patient’s stabilisation she was discharged on antibiotics for another 6 months, and then all antibiotics were discontinued. Unfortunately, a few month later she developed again malaise,
fever, lymphadenitis and laboratory signs of inflammation. Assuming a relapse of *Burkholderia cocovenenans* disease, antibiotic therapy was reapplied but achieved no clinical improvement. Microbiological diagnostics failed to detect *Burkholderia cocovenenans* in clinical specimens but in the course of disease bone marrow aspirates revealed infection with *Mycobacterium chelonae*. Broad antimycobacterial therapy was immediately initiated but the patient’s condition rapidly deteriorated and severe sepsis developed. Empirical therapy with imipenem was added to the antibiotic regimen, nevertheless, the patient died from irreversible septic shock and multiorgan failure. Since no microorganisms other than NTM were detected in blood cultures and postmortem tissue specimens from liver, spleen and lymphnodes, *Mycobacterium chelonae* was considered to be the causative agent of fatal septic shock.

*Concanavalin A stimulation.* To induce IFNγ synthesis, either whole blood cells or isolated PBMC (1x10⁶ cells/ml in RPMI 1640 medium supplemented with 10% FCS and 2 mM glutamine) were stimulated with 100 (whole blood) or with 10 (PBMC) µg/ml Concanavalin A (Serva) over 24h and IFNγ was measured in the supernatant by ELISA (Biosource).

*Plasma spiking with increasing concentrations of IFNγ.* Recombinant human IFNγ (Imukine®, Boehringer Ingelheim) was incubated with the patient’s plasma at a ratio of 1:1 and the recovery of IFNγ was measured by ELISA.

*Testing for biological relevance of the IFNγ neutralizing activity.* PBMC (1x10E6 cells/ml) from a healthy proband were incubated with 5 % (v/v) patient’s plasma or control plasma +/- recombinant human IFNγ (1 ng/ml) for 20h followed by stimulation with LPS (50 pg/ml, Sigma) for 4h. The supernatants were analyzed for TNFα (Immulite, DPC Biermann) and monocytic HLA-DR expression was measured by flowcytometry (FACS Calibur, Becton Dickinson). Data are shown as ratio of IFNγ + LPS / LPS alone.
**Anti-human IgG isotype ELISA.** Patient’s serum or control serum was added to plates coated either with recombinant human IFNγ (PeproTech Inc.) or recombinant human IL-10 (control). Following extensive washing and incubation with POD-labeled anti-human IgG isotype antibodies, the substrate reaction was measured.

**Results and discussion**

Whole blood stimulation with Concanavalin A revealed no detectable IFNγ production, whereas IL-4 production was unaffected. Intracellular IFNγ staining of whole blood cells following PMA / ionomycin stimulation was positive excluding deficient IFNγ production. Surprisingly, stimulation of PBMC with Concanavalin A showed normal levels of IFNγ secretion (all data not shown). This suggested an inhibitory IFNγ activity in the patient’s plasma. We tested this hypothesis by adding increasing concentrations of recombinant IFNγ to the patient’s plasma and measuring IFNγ concentration by ELISA (Fig. 1A). In contrast to control plasma, the patient’s plasma completely blocked the detection of IFNγ indicating an extremely high-affinity anti-IFNγ activity. This activity was specific for IFNγ as the detection of recombinant TNFα, IL-10, IL-5, IL-4 and IL-2 was not affected by the patient’s plasma (data not shown). To test the biological relevance of this finding, PBMC from a healthy volunteer were incubated with IFNγ in the presence of either patient’s plasma or control plasma and stimulated with LPS. We then measured TNFα production as well as monocytic HLA-DR expression - both parameters are normally up-regulated by IFN. In contrast to control plasma, the patient’s plasma almost completely inhibited TNFα and HLA-DR up-regulation by exogenous IFNγ (Fig. 1B).
In the absence of autologous plasma, the patient’s PBMC responded normally to exogenous and endogenous IFN\(\gamma\) excluding defects in IFN\(\gamma\) mediated signalling.

Further analysis demonstrated that plasma IgG depletion by Protein A removed the anti-IFN\(\gamma\) activity, indicating the existence of an anti-IFN\(\gamma\) auto-antibody (data not shown). The isotype was subsequently identified as IgG4 by anti-IgG isotype-specific ELISA (Fig. 2).
Preliminary results suggest that this auto-antibody recognizes an epitope in the C-terminal region of IFN-γ, but the pathogenesis of auto-antibody formation to IFNγ in this patient remains unclear. Naturally occurring anti-cytokine antibodies have been described in healthy persons and are believed to be regulatory natural auto-antibodies (11). To our knowledge, this is the first report on a natural auto-
antibody to IFNγ which exhibits such a powerful neutralizing capacity resulting in severe immunodeficiency. Based on this observation testing for IFNγ neutralizing auto-antibodies should be included in patients with recurrent infections with intracellular pathogens and especially with NTM. Tragically, the patient died due to severe sepsis before knowing the nature of the inhibitory activity. With earlier knowledge of the character of the inhibitory activity, alternate therapeutic approaches (e.g. IVIG, selective protein immunoadsorption) may have been helpful.

References


Legends to figures

Figure 1. **A. Plasma spiking of increasing concentrations of IFNg demonstrates high-affinity IFNg binding activity.** Recombinant human IFNg was added to patient and control plasma at various concentrations and plasma IFNg concentrations were determined by ELISA. **B. IFNg binding activity does neutralize IFNg activity.** PBMC from a healthy proband were incubated with patient’s plasma or control plasma with or without recombinant human IFNg for 20h followed by stimulation with LPS for 4h. The supernatants were analyzed for TNFalpha and monocytic HLA-DR expression was measured by flowcytometry. Data are shown as ratio of IFNg + LPS / LPS alone.
Figure 2. **High affinity IFNγ binding activity is an anti-IFNγ IgG4 autoantibody.** Patient’s serum (black bars) or control serum (white bars) was added to plates coated either with recombinant human IFNγ or recombinant human IL-10. Following extensive washing and incubation with POD-labeled anti-human IgG isotype antibodies, the substrate reaction was measured.
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