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Two cases illustrating successful adjunctive interferon-g immunotherapy in refractory disseminated coccidioidomycosis

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Summary

Protective immunity and host resistance to coccidioidomycosis require a robust cell-mediated immunity with adequate production of Th1 cytokines including interleukin-12, and IFN-g and appropriate regulation and coordinated functionality of Th1/Th2 responses and IL-12/IFN-g cytokine axes. IFN-g augments the anti-fungal activity of effector immune cells against a variety of fungi. Numerous animal models have demonstrated the potential efficacy of adjunctive IFN-g in treatment of invasive mycoses. Yet, despite these promising data, a paucity of literature documents efficacious adjunctive IFN-g administration in refractory coccidioidomycosis. We present two cases of refractory disease occurring at our institution who responded to adjunctive IFN-g.

Keywords

Interferon; Coccidioidomycosis; Immunotherapy; IFN-g; IL-12

Introduction

Coccidioidomycosis encompasses two unique species with similar clinical presentations: *Coccidioides immitis* which is endemic to arid and semiarid desert regions of central California and southern Arizona; and *Coccidioides posadasii* in southern Arizona, southwestern New Mexico, and west Texas. Symptomatic cases (40% of exposed) typically manifest as a self-limiting pneumonia (incubation period: 10e16 days). One-third of the cases of community acquired pneumonia may be due to coccidioidomycosis in Tucson,

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Conflict of interest

The authors have no financial or commercial interest in this work or the immune mutational analyses or treatment interventions evaluated.

Arizona¹. However, 5% of exposed individuals suffer extra-pulmonary dissemination, most commonly involving cutaneous, skeletal, and central nervous system systems, invariably requiring prolonged anti-fungal therapy.²

Protective immunity, and disease resistance requires a robust cell-mediated immunity (CMI), Th1 and IgG2a antibody-subclass immune bias, and appropriate coordination and regulation of Th1/Th2 responses and interleukin-12/IL-23/IFN- γ axes. Humoral immunity plays a lesser role, evidenced by failure of passive antibody transfer providing protection against disease,¹ akin to other intracellular infections.^{3,4} IFN- γ has pleiotropic adjunctive effects on cellular defense including stimulation of B-cells (antibody production), T-cells (promoting Th1-bias), neutrophils (promoting survival), and phagocytes (activation potentiates granuloma formation and microbicidal and tumoricidal activity). IFN- γ release promotes Th1-biased cytokine induction (notably including TNF- α), production of reactive oxygen and nitrogen species, and elevated MHC-class II antigen processing and presentation. IFN- γ augments the anti-fungal activity of effector immune cells, and animal models have consistently demonstrated the potential efficacy of adjunctive IFN- γ in treatment of invasive mycoses. Adjunctive IFN- γ was effective in a host of infectious diseases including mycobacterial, and cryptococcal infections stemming in part from mutational defects within the IL-12/IFN- γ axis. Known mutations have been identified within the IFN- γ and IL-12 receptors (IFNGR1, IFNGR2, IL-12Rb1), and the STAT1 (signal transducer and activator of transcription) protein. Mutations affecting IFN- γ R1 and IL-12Rb1 have been linked to severe histoplasmosis, and paracoccidioidomycosis, respectively.⁴ The mutation 818del4 within the IFN- γ R was recently identified in a patient experiencing recalcitrant disseminated coccidioidomycosis,⁴ and a missense mutation (C186Y) in IL-12Rb1 was recently described in two patients (siblings) with disseminated coccidioidomycosis.⁵ Adjunctive IFN- γ immunotherapy was successfully utilized as salvage therapy in refractory coccidioidomycosis in two previous cases.⁴

We present two cases of refractory disease, which responded clinically to adjunctive IFN- γ . As there is a dearth of publications discussing the efficacy of IFN- γ immunotherapy in the treatment of refractory coccidioidomycosis, and as its use remains anecdotal, we believe that it is important to add our experience to the literature. In addition to evaluating the clinical response to this intervention, we pursued an in-vitro evaluation of the IFN- γ /IL-12 pathway. Screening for potential carriage of known pathway mutations, coupled to an analysis of in-vitro cytokine expression may provide insight into the underlying immunological correlates yielding a poor clinical response, as well as susceptibility to severe and disseminated disease. This testing may ultimately prove useful in endemic regions, as part of screening, and surveillance campaigns [identifying vulnerable individuals susceptible to severe, and disseminated disease (perhaps via their in-vitro response to coccidioidal-antigen mediated interferon- γ release assays, and/or expansive Th1/Th2 cytokine profiling) potentiating prophylactic interventions], in addition to its potential role in the clinical arena. Additionally, it is paramount to prognosticating the potential efficacy in adjunctive immunotherapy, providing support for exploiting such a hitherto empiric intervention.

Methods

We administered 100-mcg IFN- γ injections three-times weekly (50 mg/m² body-surface-area dosing as reported in disseminated mycobacteria and chronic granulomatous disease).⁴ This dosing strategy proved efficacious in refractory disseminated mycobacteria infections unresponsive to treatment after three months.⁶ The CF-titers were performed by Dr. Pappagianis' laboratory at the University of California-Davis School of Medicine. All other testing was performed by the national institutes of health (NIH) Immunology Flow Cytometry laboratory certified under the Clinical Laboratory Improvement Amendment of 1988 as qualified to perform high complexity clinical laboratory testing under the auspices of the National Institute of Allergy and Infectious Diseases, under study title: Genetic Analysis of Immune Disorders; study number 95-I-0066. The patients signed an informed consent through the NIH IRB.

Lymphocytic phenotypic and cytokine-stimulation testing was facilitated by acquiring 5 cc/30 cc of blood drawn into EDTA and sodium heparin tubes, respectively. DNA sequence analyses were performed via complementary DNA (cDNA) with total RNA extracted from PBMC in RNazol B (Tel-Test, Friendswood, TX). Complementary DNA was synthesized using SuperScript II RNase H-reverse transcriptase (Life Technologies) according to the manufacturer's instructions. The open reading frame of the IFN γ R1 gene was amplified by PCR with primers 5⁰GTTGGAGCCAGC GACCGTCGGTA3⁰ and 5⁰CCAGGAAAATCAGACTTCAAAG3⁰,^{7,8} while the open reading frame of the IFN γ R2 gene was amplified by PCR with primers 5⁰ACCTGAGCCGCCGCCGAGCG3⁰ and 5⁰TCCGATGGCTTGATCTCTTC3⁰ designed from published sequence data.⁸ Exon III and portions of the flanking introns of IFN γ R2 were amplified by PCR with primers.

5⁰CTGCAGGAATTCTGTGAATTG3⁰ and 5⁰CTCTTACCATTCCGATAGTG3⁰.^{7,8} PCR products were purified with the Wizard PCR Prep kit (Promega Inc., Madison, WI), and then sequenced bidirectionally with the Sequenase CyclistTM Taq DNA Sequencing kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions.

We used flow cytometry to identify T-cell subsets, and activation status. Fluorescence-activated cell sorting was performed with peripheral blood anticoagulated with EDTA analyzed on a FACS can flow cytometer (Becton Dickinson, San Jose, CA). The monocyte gate was determined with anti-CD14 antibodies (PharMingen, San Diego), then back-gating with forward and side scatter to determine monocytes. CD14 is the endotoxin receptor molecule on the surface of phagocytes. Anti-IFN- γ R1 antibody (1224-00; Genzyme) was used unconjugated with a goat anti-mouse antibody labeled with fluorescein isothiocyanate (Caltag, San Francisco). Anti-CD3 (Becton Dickinson), anti-CD4 (Coulter Immunotech, Westbrook, ME), anti-CD16/56, and anti-CD20 (Becton Dickinson) were directly conjugated.

Peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation of heparinized whole blood through lymphocyte separation gradient (BioWhittaker, Walkersville, MD), and then washed with Hanks' balanced salt solution. PBMC (10⁶/mL) were cultured in RPMI medium at 10⁶ cells per milliliter in microtiter plates in separate

wells for cytokine-stimulation studies. This involved assaying for cell culture supernatants of IL-2, IL-12p70, IL-1b, IFN- γ , IL-6, IL-10, TNF- α , and granulocyte monocyte colony stimulating factor (GM-CSF) at baseline, and upon stimulation with phytohemagglutinin (PHA (1%) e Sigma, St. Louis MO, USA), PHA β IL-12, IL-12 (10ng/ml), IFN- γ (1000U/ml e R&D Systems, Minneapolis, MN, USA), lipopolysaccharide (LPS) (200ng/ml e Sigma, St Louis, MO, USA), LPS β IFN- γ , IL-1b (100 ng/ml), phorbol myristate acetate (PMA) (100 ng/ml) β ionomycin (ION) (1mM), segmental antigen challenge with Staphylococcal aureus Cowan Antigen (SAC (0.01%) e Pansorbin; Calbiochem San Diego), and IL-6 (100ng/ml). Stimulation lasted 48 hs at 37 °C, with the supernatant then frozen at -20 °C until cytokine determination with commercial ELISA kits as specified by the manufacturer.

Case 1

A 30-year-old previously healthy African-American active-duty Navy male was diagnosed with disseminated coccidiomycosis while stationed at Naval Air Station (NAS) Lemoore, California. Past medical history was significant for alcohol dependence in remission for 3 years, negative HIV-status, and latent tuberculosis (completed 9-months isoniazid in 2007). He denied taking medications, herbals, illicit, tobacco or current alcohol use. His initial presentation included a non-productive cough, dyspnea, fatigue, night sweats, myalgias, and arthralgias. Examination was significant only for tachycardia (110 beats/minute). He was initially diagnosed with a viral syndrome (nasopharyngeal swab negative for influenza). Two-weeks later he returned, his symptoms unabated. Additionally, he developed right foot and low back pain, and disseminated cutaneous lesions (7e8 mm nodules; non-tender, and non-pruritic). Labs were significant for leukocytosis (18,900 cells/mm³ and 11% eosinophilia), mild transaminase elevation (AST 48 U/L, ALT 100 U/L), elevated sedimentation rate (ESR) [>120], elevated c-reactive protein (CRP) [24 mg/dL], negative HIV serology and positive coccidioides serology (the ELISA was positive for both IgG and IgM, and presenting complement-fixation (CF) titer was 1:8). Titers less than or equal to 1:2 are considered negative.

Two cutaneous lesions were biopsied, both growing *C. immitis*. Chest radiography was normal. Chest computed tomographic (CT) imaging revealed reticulonodular disease. Systemic MRI evaluation revealed osteomyelitis of his right foot; and lesions involving the vertebral bodies of the thoracic, and lumbar spine, sacrum, bilateral iliac wings, ribs, skull, and long bones of the legs bilaterally. Treatment was initiated with liposomal amphotericin and posaconazole (400-mg bid) with fatty meals. Therapeutic serum drug concentrations confirmed satisfactory absorption. Amphotericin was discontinued after two-months given pronounced electrolyte wasting and renal insufficiency, while continuing posaconazole. At that time, skin lesions faded, foot pain resolved, but back pain persisted. His fevers, fatigue, coughing, and night sweats persisted albeit improved. MRI evaluation of his thoracic-lumbar spine at this time revealed slight disease progression, and CF-titers were 1:64. After an additional 4-weeks (now 12-weeks since presentation), there was no further clinical improvement, and the CF-titer increased to 1:128. Posaconazole was then administered at 200-mg qid (exploiting superior absorption), thence 240-mg qid. After 5 additional months on this regimen, he failed to exhibit significant clinical, laboratory (CF-titer remained at

1:128), or radiographic improvement. Additionally, his inflammatory markers (ESR and CRP) remained elevated at 105 and 5.0, respectively.

Case 2

A 23-year-old previously healthy African-American Active-Duty male stationed at NAS Lemoore, California developed severe unremitting back pain localized to the thoracic and lumbar spine, initially responding to non-steroidal anti-inflammatory analgesics. Plain films were unremarkable. He denied prior medical, or surgical history, nor taking medications, tobacco, illicit, or alcohol. Pain progressed over the ensuing weeks, refractory to analgesics, and then accompanied by fevers, night sweats, and unintentional (15-pound) weight loss prompting further evaluation. Labs were significant for 7% eosinophilia, negative HIV serology, and a positive coccidioides serology (ELISA positive for both IgG and IgM and a CF-titer of 1:64).

An MRI revealed lesions consistent with disseminated coccidioidomycosis with involvement of the cervical, thoracic and lumbar spine, the iliac crests, and ribs. A bone biopsy was culture-positive for *C. immitis*. Induction therapy was initiated with liposomal amphotericin-B at 5 mg/kg/day and oral itraconazole. After several weeks, his back pain worsened, and imaging demonstrated progression of the bone lesions accompanied by L3 vertebral-body collapse requiring corpectomy. His CF-titer remained at 1:64. Itraconazole was switched to posaconazole (continuing amphotericin). Despite maximizing amphotericin (10 mg/kg) and posaconazole (therapeutic serum drug concentrations repeatedly confirming satisfactory absorption), he clinically worsened developing new neurologic deficits (clonus and lower extremity weakness). Four-weeks after switching to posaconazole his CF-titer increased to 1:512, and after 4 months further, (5 months from commencing posaconazole) it peaked at 1:1024. MRI imaging revealed lesion progression (vertebral cortices involvement, epidural extension, and retropulsion of T10 fractured fragments into the spinal canal endangering cord compromise). He subsequently underwent a T12 vertebroplasty and T10 kyphoplasty. His weight declined from 170 to 108 pounds over this interval.

Both cases were characterized by a lack of significant clinical, laboratory, and radiographic improvement (Case 1) and frank worsening (Case 2), despite prolonged maximal optimally tolerated chemotherapy.

Results

Clinical

Both patients exhibited minimal injection site reactions, and mild influenza like symptoms manifest by fever, chills, and fatigue which subsided upon continuation of therapy beyond a week.

Case 1—After 6 months of IFN- γ immunotherapy (thence discontinued), surveillance MRI imaging revealed improvement in lesions. His CF-titer remained at 1:128, while inflammatory markers decreased significantly within several weeks of treatment (ESR and CRP 41 and 1.3, respectively) normalizing by 4 months (18/0.4). Additionally, the patient consistently conveyed improvement in symptoms, manifest by decreasing back pain, and

fatigue exceeding any gradations in improvement experienced hitherto facilitating increasing exercise capacity and return to work.

Case 2—Two-weeks after initiation of IFN-g therapy he noted markedly decreased bone pain, and resolution of neurological deficits. Ambulation markedly improved, and weight increased. His CF-titers declined to 1:128 after 3 months; and ESR and CRP normalized to 17 and 0.9, respectively. Surveillance MRI imaging revealed stable lesions. After significant clinical, radiographic, and laboratory improvement upon 6 months of IFN-g treatment (CF-titers stable at 1:128), it was discontinued. After a 4-month hiatus from IFN-g (remaining on posaconazole) he noted incrementally increased back pain. MRI imaging revealed lesion progression; ESR and CRP increased to 89 and 6.5 respectively; and CF-titer remained 1:128. IFN-g was restarted, and after 4 months, he experienced repeated improvement in clinical symptoms, with MRI imaging revealing stability in bone lesions and regression in soft-tissue involvement. His CF-titer decreased to 1:32 and inflammatory markers improved again to 20/1.98.

Laboratory: DNA sequencing, lymphocyte phenotyping and cytokine-stimulation responses

For both patients, immunofluorescence for the IFN-g receptor-1 on monocytes was normal as was DNA sequencing of IFN-g receptor-1 (IFN-gR1), IFN-gR2, and STAT1, with normal production of analytes upon stimulation with toll-like receptor (TLR) agonists (LPS and IL-1 e suggesting normal IRAQ-4/MYD88 function). Table 1 depicts the total T-cells and T-cell subsets which reveal no striking anomalies for both cases. The salient data to draw from the cytokine-stimulation data (Table 2 e to be commented upon in the Discussion section) involves: (1) the response of IFN-g and TNF-a to PHA (1%), with/without IL-12 [IFN-g increased from 12768.4 to 42323.7 in Case 1 and from 40452.9 to 107751.6 in Case 2; while TNF-a decreased from 6808.0 to in Case 1 and increased from 6694.0 to 9087.2 in Case 2]; and (2) the TNF-a response to LPS (200 ng/ml) with/without IFN-g [decreased from 2200.2 to 2100.0 in Case 1; and increased from 1485.8 to 4001.8 in Case 2] (highlighted).

Discussion

The most salient aspect of this report is the dramatic subjective and objective clinical responses when IFN-g was added to both patients' treatment regimens, while demonstrating minimal side effects. Moreover, repeated clinical deterioration by Case 2 after treatment cessation, and clinical improvement upon re-introduction of IFN-g buttressed support for its efficacy.

There was no quantitative abnormality in peripheral blood CD3^b T-cells and subsets (Table 1), nor within the CD14^b monocytes, CD20^b B-cells, and CD16^b natural killer (NK) cells (data not shown). We identified normal cellular capacity to proliferate signaling [via phytohemagglutinin (PHA), lipopolysaccharide (LPS), phorbol myristate acetate and ionomycin (essentially bypasses the IFN-g receptor assembly), and Staphylococcal cowan antigen induced escalations in TNF-a and IFN-g] suggesting normal functionality of cytokine expression machinery, and innate immune pathways. We identified normal augmentation of IL-12 via PHA, and LPS, and normal DNA sequencing of IFN-gR1, IFN-

gR2, and STAT1. The IL-12 receptor was not sequenced, mutations in which have been associated with coccidioidomycosis and mycobacterial disease.⁵ However, the normal increase in IFN-g to IL-12 suggests this sequencing will be uninformative. The most compelling results identified in this cytokine analysis is the blunted augmentation of TNF- α production with the introduction of the combinations (PHA $\pm$ IL-12), and (IFN-g $\pm$ LPS). Inferences cannot be drawn necessarily from absolute cytokine values, which are composites of multi-factorial influences. We may, however, derive meaningful interpretations of the relative increases in cytokine responses under various stimuli (combinations of stimuli) as compared to population norms. In Case 1, the increased ratio of IFN-g expression under stimulation from PHA coupled to IL-12, as compared with the isolated PHA stimulus alone was 3.3 (expected ratio $\approx$ 3.79). TNF- α expression decreased under stimulation with PHA $\pm$ IL-12 as compared to the isolated PHA stimulus alone, (expected increased ratio response $\approx$ 1.52). Similarly, TNF- α expression decreased under stimulation with LPS coupled to IFN-g as compared to isolated LPS stimulus alone, (expected increased ratio response $\approx$ 4.3). In Case 2, the increased ratio of IFN-g expression under stimulation from PHA coupled to IL-12, as compared with the isolated PHA stimulus alone was 2.66 (expected ratio $\approx$ 3.79). TNF- α expression increased under stimulation with PHA $\pm$ IL-12 as compared to the isolated PHA stimulus alone (increased ratio $\approx$ 1.36 compared to an expected 1.52). Finally, TNF- α expression increased under stimulation with LPS coupled to IFN-g as compared to isolated LPS stimulus alone (increased ratio $\approx$ 2.69 compared to an expected 4.3). Therefore, we identified a possible defect within the IFN-g signaling pathway, the precise molecular defect hitherto unidentified (and potentially different between both cases), and possibly compensated with supra-physiologic/supra-therapeutic IFN-g dosing.

There are two components to the IFN-receptor, IFN-gR1 and IFN-gR2. After successful ligand binding, the intracellular portion of both IFN-g receptors participate in a complex subsequently activating (via phosphorylation) JAK1/2 tyrosine kinase, and STAT1 which trans-locates to the nucleus to bind IFN-g activating sequences inducing expression of hundreds of genes including TNF- α .⁹ Known mutations within this pathway (IFNgR1, IFNgR2, IL12RB1, IL12Rp40, and STAT1) are identified in Mendelian susceptibility to mycobacterial disease, as well as refractory cases of coccidioidomycosis as described in the Introduction.^{2,10} Additional immune deficiencies result from cytokine auto-antibodies (notably IFN-g) increasingly recognized as culpable for inferior host immunity.^{2,3} An early over-exuberant activity of Th2 cytokines (i.e., IL-10 and IL-4 which are integral to immunomodulation, immunoregulation, and regulatory T-cell stimulation), is deleterious,¹¹ as the reciprocal mitigation of an early exuberant Th1-biased inflammatory response undermines optimal CMI.¹¹ Corroborating these assertions, overt depression of humoral immunity confers disease resistance, and African-Americans and Filipinos known to be at elevated risk for disseminated infection manifest a Th2 immune bias.¹¹ Generalizing, immune-dysregulation manifest by suboptimal timing, duration, strength, coordination, and relative immune bias may be instrumental in disease susceptibility to numerous intracellular pathogens including coccidioidomycosis. Further research into discrete mutations, and functional single-nucleotide-polymorphisms of the proteins comprising the IL-12/IL-23/IFN-g axis, and cytokine responses should provide useful prognostic information, risk stratification, and insight into potential treatment algorithms. For example, salient

investigations include identifying cytokine profiles predicting resistance to severe and/or disseminated coccidioidomycosis. Indeed, functional polymorphisms in genes involved within the IL-12/IL-23/IFN- γ axis may account for the genetic predisposition to severe disease.⁵

Immunotherapy has not engendered superior outcomes nor is economically practical on a population-based perspective. However, targeted genetic testing of the IL-12/IFN- γ pathway in individuals exhibiting severe, and/or prolonged manifestations and inferior treatment response to infectious diseases requiring robust host CMI could prove cost-effective. This is analogous to targeted pharmacogenetic testing shown to optimize individual pharmacotherapy predicting host-specific pharmacodynamics, drug-interactions, and toxicities.

Our two patients demonstrated blunted IFN- γ mediated increases in cytokine production suggesting a defect isolated to intracellular signaling, or gene transcription, and translation. Intuitively, mutations leading to dysfunctional IFN- γ production including suboptimal receptor binding, and intracellular signaling may be compensated, or frankly overcome with supra-physiologic/supra-therapeutic dosing of IFN- γ .⁸ Although we cannot prove IFN- γ produced improved clinical results, the biologic plausibility and the temporal association is compelling.

As this is essentially a retrospective observational study of two patients, interpretations are limited. One notable limitation in our interpretation is that the immunological analysis occurred several months after disease presentation, (about 8 months, and 5 months in Case 1 and Case 2, respectively) and after institution of therapy which undoubtedly influences the host immune responses. Further study of potential abnormalities contributing to suboptimal IL-12/IL-23/IFN- γ axis function may include characterization of (1) STAT1 dimerization, nuclear translocation, and induction of IFN- γ activating sequences; (2) X-linked nuclear factor- κ B essential modulator (NEMO); and (3) JAK-1/JAK-2 function. Additional study could address optimal timing and dosing of IFN- γ , and other immunotherapeutic Th1 and Th2 cytokines, dictated by results of immunological analysis.

In conclusion, we advocate concerted consideration of early IFN- γ adjunctive therapy, with or without IL-12/IFN- γ axis mutational screening, in selected cases of refractory disseminated coccidioidomycosis infections.

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Table 1Lymphocyte phenotype report.^a

Parameter	Case 1 ^b		Case 2 ^c	
	Absolute number (controle 95% CI)	% (controle 95% CI)	Absolute number (controle 95% CI)	% (controle 95% CI)
<i>Total T-cells</i>				
CD3	706 (714e2266)	88.2 (60e83.7)	797 (650e2108)	79.7 (57.3e86.4)
CD3/alpha-beta	671 (675e2235)	83.9 (55.8e80.1)	783 (659e1812)	78.3 (54.2e76.5)
CD3/gamma-delta	35 (8e261)	4.3 (0.5e10.4)	14 (9e163)	1.4 (0.7e8.8)
<i>T-cells subsets</i>				
CD4 ^b /CD3 ^b	419 (359e1565)	52.4 (31.9e62.2)	440 (358e1259)	44.0 (28.6e57.2)
CD8 ^b /CD3 ^b	253 (178e853)	31.6 (11.2e34.8)	334 (194e836)	33.4 (12.9e46.9)
CD4/CD8 ratio	1.66	1.11e5.17	1.32	0.74e3.58
CD4 ⁺ CD8 ⁻ /CD3 ^b	28 (18e185)	3.5 (1.3e9.2)	18.0 (12e102)	1.8 (0.9e5.4)
CD8 ^b CD57 ^b /CD3 ^b	189 (< 397)	23.6 (<16.2)	212 (<239)	21.2 (<15.8)

^aTest developed and performance characteristics determined by NIH Immunology Flow Cytometry Laboratory. This Lab is certified under the Clinical Laboratory Improvement Amendments of 1988 as qualified to perform high complexity clinical laboratory testing.

^bComplete blood count: Total leukocyte count of 5.1×10^3 cells/mm³ with a hematocrit of 43.5% and 250/mm³ platelets.

^cComplete blood count: total leukocyte count of 8.0×10^3 cells/mm³ with a hematocrit of 32.1% and 441/mm³ platelets.

Table 2

Cytokine-stimulation analysis.^{a,b,c}

Stimulating input	Case 1					Case 2					IL-6	IL-10	TNF- α	IFN- γ	IL-1b	IL-12p70	IL-2	IL-12p70	IL-1b	IFN- γ	IL-6	IL-10	TNF- α	IFN- γ	IL-1b	IL-12p70	IL-2	IL-12p70	IL-1b	IFN- γ	IL-6	IL-10	TNF- α																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
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Media	26.9	22.1	10.9	39.1	2.5	8.2	8.7	16.6	11.0	5.4	67.4	11.5	11.0	16.4																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													

PHA: phytohemagglutinin; PMA: phorbol ester-phorbol myristate acetate; ION: ionomycin; SAC: segmental antigen challenge, *Staphylococcus aureus* Cowan Antigen.^aTest developed and performance characteristics determined by NIH Immunology Flow Cytometry Laboratory. This Lab is certified under the Clinical Laboratory Improvement Amendments of 1988 as qualified to perform high complexity clinical laboratory testing.^bCytokines are measured in pg/ml.^cThe salient data to draw from the cytokine-stimulation data involves the response of IFN- γ and TNF- α to PHA(1%), with/without IL-12; and the TNF- α response to LPS(200 ng/ml) with/without IFN- γ .