A novel insight into the immunologic basis of chronic granulomatous invasive fungal rhinosinusitis

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ABSTRACT

Background: *Chronic granulomatous invasive fungal rhinosinusitis (CGIFRS) is a rare disease. The underlying immune responses that drive the development of CGIFRS, as opposed to successful pathogen clearance and controlled inflammation, are not currently known.*

Objective: *To characterize the immune responses associated with CGIFRS.*

Methods: *In addition to a battery of basic investigations, more in-depth immunologic testing involves ex vivo whole-blood stimulation with the polyclonal T-cell mitogen phytohemagglutinin and fungal antigens with interleukin (IL) 12, was undertaken to investigate cell-mediated immune responses associated with CGIFRS.*

Results: *Ex vivo whole-blood stimulation with the polyclonal T-cell mitogen phytohemagglutinin and fungal antigens with IL-12 identified reduced interferon gamma and increased IL-17A levels within the supernatant, which indicated increased in vivo T-helper (Th)17 responses and impaired Th1 responses compared with healthy controls.*

Conclusion: *These findings suggest that the development of CGIFRS may be associated with an abnormally exaggerated host Th17 response, which caused failure to clear the fungal pathogen with refractory fungal infection of mucosal membranes, resulting in chronic tissue inflammation.*

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Based on histopathologic features, five categories of fungal rhinosinusitis are currently recognized. These can broadly be divided into tissue-invasive and noninvasive categories. The tissue-invasive category includes acute necrotizing (fulminant) fungal rhinosinusitis, chronic invasive fungal rhinosinusitis, and granulomatous invasive (indolent) fungal rhinosinusitis. The noninvasive category includes fungal ball (sinus mycetoma) and allergic fungal rhinosinusitis (eosinophilic mucin rhinosinusitis).[1]

The granulomatous subtype of chronic invasive fungal rhinosinusitis is rare, with an unknown immunologic basis. Chronic granulomatous invasive fungal rhinosinusitis (CGIFRS) commonly affects a host who is immunocompetent, with *Aspergillus flavus* being the most common causative agent reported most frequently in subtropical areas of Sudan, Saudi Arabia, India, and Pakistan.[1] Clinically, CGIFRS can present as an enlarging mass that affects the cheek, orbit, nose, and paranasal sinuses,[2] characterized on histopathologic examination by noncaseating granulomas with foreign body or Langerhans giant cells and considerable fibrosis.[3] CGIFRS poses a significant diagnostic and management challenge for the clinician due to its rarity, unclear pathogenesis, and the lack of agreed management protocols.[4]

A 37-year-old white woman of Canadian origin presented with a 5-month history of symptoms suggestive of chronic rhinosinusitis, including nasal obstruction, anosmia, and crusting. She was otherwise fit and well, with no medical history suggestive of immunodeficiency. On examination, there was a widened nasal bridge, with tenderness over the nasal bones, crusting within the nasal cavities, and some mucopus in the middle meati. She was initially treated for chronic rhinosinusitis with 2 months of maximal medical therapy, including clarithromycin, topical nasal steroids, and

Figure 1. *(a) Coronal T1-weighted magnetic resonance imaging (MRI) at presentation, showing low-intensity concentric mucosal thickening mainly within the left nasal cavity consistent with fungal disease. (b) Coronal T1-weighted MRI 1 year after surgery, showing satisfactory appearances, with no radiologic evidence of recurrent disease.*
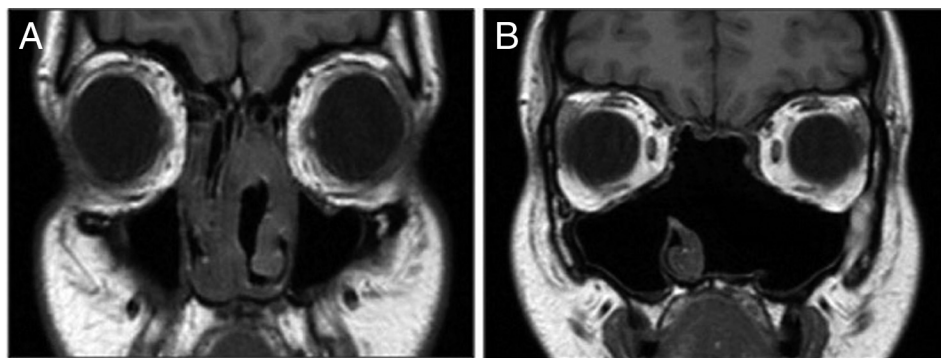
Figure 2. *Axial computed tomography of the paranasal sinuses, showing demineralization and irregular erosion of the nasal bones.*

nasal saline solution douching. Despite this, she remained symptomatic, and imaging of the sinuses, which included computed tomography and magnetic resonance imaging (Fig. 1 *a*) demonstrated widespread mucosal thickening within the sinuses as well as evidence of significant nasal bone (Fig. 2) and septal erosion.

A nasal examination with the patient under general anesthesia was scheduled and showed widening of the nasal bridge (Fig. 3*a*), with multiple whitish submucosal lesions, mainly on the left side, which involved the anterior aspect of the nasal septum and lateral nasal wall (Fig. 3 *b*), with a degree of crusting. The affected areas were biopsied, and histologic examination revealed evidence of granulomas and multinucleate giant cells, with clear evidence of fungal hyphae and spores (Fig. 4). On this basis, a diagnosis of CGIFRS was made. The fungus was later identified as an *Alternaria* species, susceptible to all antifungals. To our knowledge, this is the first reported case of *Alternaria*-related CGIFRS. Thoracic imaging and bronchoalveolar lavage did not identify evidence of disseminated lower airway disease.

METHODS

Ethical approval was not deemed necessary because this was a single case report. Informed consent for all immunologic investigations was obtained from the patient. Nephelometry was used to quantify immunoglobulin G (IgG), IgA, and IgM levels in serum by using the IMMAGE Immunochemistry Systems (Beckman Coulter Inc., Brea, CA). Subsequent serum protein electrophoresis was performed with a gel run on Serbia Hydrasys (Sebia SA, Evry, France) by using the Hydragel protein (E) 15/30 gel kit (Sebia). Autoantibody screening for antinuclear antibodies was performed according to manufacturer's instructions by using ORG-j200 Antinuclear Antibodies Detect (Orgentec Diagnostika, Mainz, Germany) and was analyzed on Phadia 250 (Thermo Scientific, Tewksbury, MA). Antineutrophil cytoplasmic antibody was performed, according to the manufacturer's instructions, with NOVA Lite Antineutrophil Cytoplasmic Antibody Kits/Substrate Slides (Inova Diagnostics, San Diego, CA), with analysis by light microscopy inspection of fluorescence pattern staining.

Neutrophil oxidative burst was performed by using Phagoburst (Orpegen Pharma, Heidelberg, Germany) by using dihydrorhodamine-123 as fluorogenic substrate after stimulation with phorbol 12-myristate 13-acetate for 30 minutes at 37°C according to manufacturer's instructions. Lymphocyte subset analysis was performed on fresh EDTA blood by using a six-color flow cytometry panel for T-, B- and natural killer-cell immunophenotyping with monoclonal antibodies against surface antigens CD45, CD3, CD4, CD8, CD16/56$^+$, CD19 (BD Biosciences, San Jose, CA). Lymphocytes were gated on side scatter and CD45$^+$ population. Absolute lymphocyte counts were generated by dual platform analysis by using BD FACSCanto II (BD BioSciences).

Cytokine production was performed by dilution of lithium heparinized whole blood 1:5 with RPMI medium into 96-well plates and activated by either single or costimulation with 10 μg/mL of phytohemagglutinin (Sigma-Aldrich, St Louis, MO), 20 ng/mL interleukin (IL) 12 (R&D Systems, Abingdon, U.K.), and β
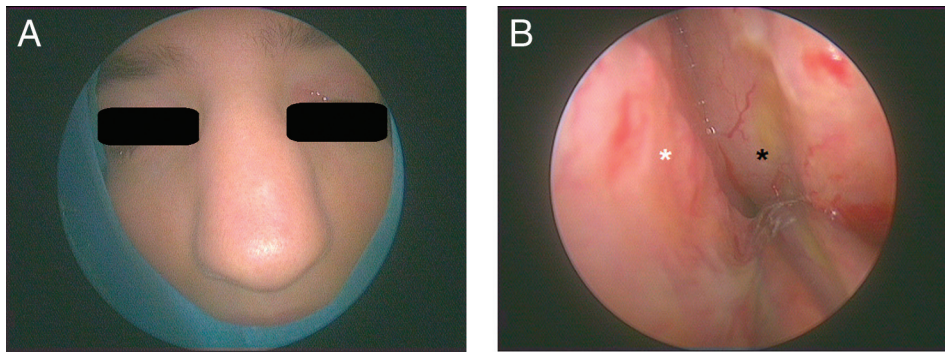
Figure 3. (a) Clinical photograph, demonstrating widening of the nasal bridge. (b) An endoscopic photograph of left nasal cavity, demonstrating whitish submucosal lesions, with prominent mucosal telangiectasia on the anterior nasal septum (white asterisk) and the lateral nasal wall (black asterisk).
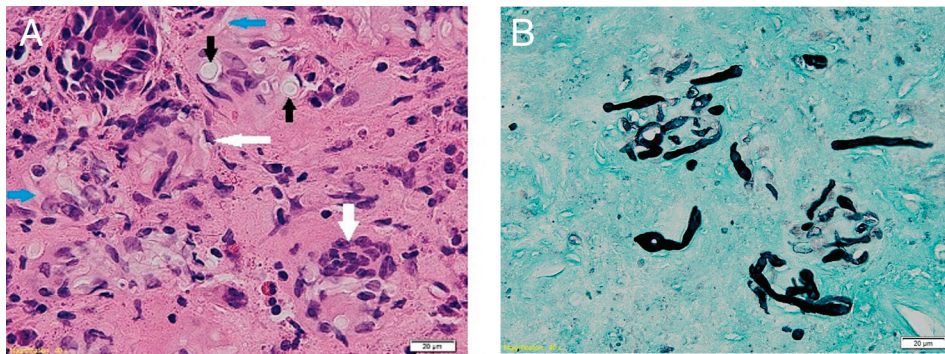
Figure 4. (a) Photomicrograph, showing giant cell granulomas (white arrows) with numerous fungal spores (black arrows) and hyphae (blue arrows) (hematoxylin and eosin, original magnification ×40). (b) Photomicrograph, showing fungal hyphae within giant cells staining positively with Grocott stain (original magnification ×40).
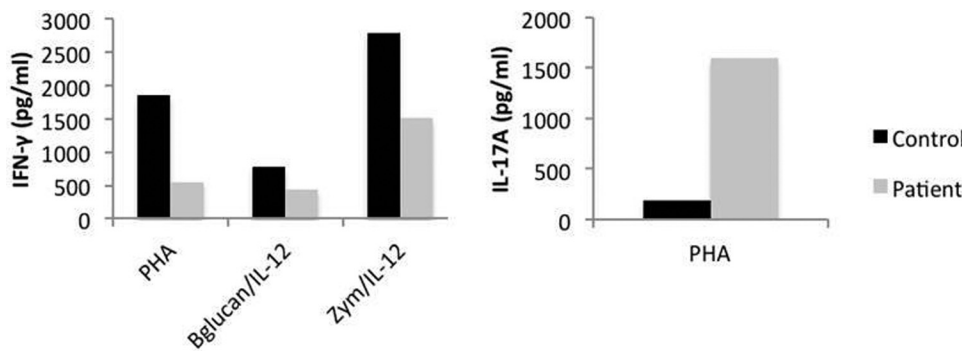
Figure 5. Ex vivo whole-blood interferon γ and interleukin 17A production in response to T-cell mitogen and fungal stimulants versus healthy control. PHA = Phytohemagglutinin; Zym = zymosan.

glucan or zymosan. Supernatants were taken at 24 hours. Cytokines were measured by using PeliKine for interferon (IFN) γ (Sanquin, Amsterdam, the Netherlands) and multiplex particle-based flow cytometry by using Fluorokine MAP for IL-17 (R&D Systems, Abingdon, U.K.).

RESULTS

Immunologic investigations revealed normal lymphocyte subsets. Total lymphocytes count was 1400 cells/mm^3: 73.2% CD3$^+$ cells, 56.6% CD3$^+$CD4$^+$ cells, 13.8% CD3$^+$CD8$^+$ cells, 18.4% CD19$^+$ cells, and 5.2% CD16/56$^+$ cells. Immunoglobulin levels were all within the normal range with IgG, 10.9 g/L; IgA, 0.9 g/L; IgM, 0.5 g/L; and IgE, 45.2 IU/mL. Neutrophil dihydrorhodamine fluorescent analysis by flow cytometry was normal, with a mean fluorescence intensity shift from −12 to 1525 in neutrophils after stimulation. Results of autoimmune antibody testing with antinu-

clear antibodies and antineutrophil cytoplasmic antibodies were negative. *Ex vivo* whole-blood stimulation with the polyclonal T-cell mitogen phytohemagglutinin, and fungal antigens β glucan or zymosan with IL-12, identified reduced IFN-γ and increased IL-17A levels within the supernatant, which indicated increased *in vivo* T-helper (Th) 17 responses and impaired Th1 responses compared with a healthy control (Fig. 5).

DISCUSSION

After discussion in a multidisciplinary team setting, including immunology, microbiology, and radiology consultants' input, and a comprehensive literature search on the subject, a decision was made to treat the patient with a combination of radical surgical resection of all macroscopically involved sinonasal tissue, antifungal treatment (a 2-week course of intravenous amphotericin, followed by a 6-month course of oral vori-

conazole), and a 6-month course of adjuvant IFN-γ 100 mcg three times weekly. A further endoscopic examination of the nose, with biopsies, with the patient under general anesthesia was undertaken 6 months after the initial surgery at the end of the treatment period with antifungals and IFN-γ, which revealed normal macroscopic appearances, with histology of the biopsy specimens that revealed inflammatory changes only, with no evidence of recurrence of the fungus. Magnetic resonance imaging 1 year after surgery remained normal (Fig. 1 *b*). The patient remained asymptomatic and disease-free 3 years after initial presentation, with planned ongoing interval endoscopic and radiologic monitoring.

The underlying immune responses that drive the development of CGIFRS as opposed to successful pathogen clearance and controlled inflammation are not known. Immunity to fungal pathogens is orchestrated by distinctive functions of Th cells. The differentiation of naive T cells to a particular Th subset is directed by dendritic cells and depends on the pattern recognition receptor signaling received by dendritic cells and their subsequent cytokine profile released.[5] Primary immunodeficiencies with impairment of the Th17 function demonstrate the need for Th17 cells in mucosal fungal immunity.[6] As such, it has become the accepted dogma that Th17 cells are vital for successful mucosal protection from fungal disease.

However, after initial fungal infection of a mucosal surface, the failure to downregulate Th17 in favor of a Th1 response results in increased localized inflammation in excess of that required to successfully clear a fungal pathogen.[5] This persisting Th17 phenotype can antagonize Th1 development and result in excessive chemoattraction of polymorphic cells, causes localized mucosal inflammation and failure to clear an invasive fungal infection.[7] This pattern of inflammation correlated with the *ex vivo* observation of increased IL-17A production in this case and the histologic appearances of CGIFRS, with continued mucosal inflammation and rich polymorphic cell infiltrate. This case indicated that the development of CGIFRS may be driven by an acquired T-cell dysregulation in patients, caused by excessive and prolonged Th17 responses at the expense of Th1 and successful fungal clearance.

Recombinant IFN-γ therapy is licensed for chronic granulomatous disease, a monogenic condition in which patients experience invasive fungal disease due to defective neutrophil oxidative burst. In chronic granulomatous disease, adjuvant IFN-γ has been shown to significantly reduce infections. In CGIFRS, adjuvant treatment with IFN-γ may help to augment the Th1 responses, modulate and antagonize Th17 cells, and lead to improved fungal immunity provided by activated innate immune cells in a similar mechanism to that in patients with chronic granulomatous disease. A limitation of *ex vivo* cytokine analysis is that whole-blood stimulation can be associated with variability both among healthy individuals as well as different assay runs. As such, extrapolation to i*n vivo* responses may not be representative.[8] However, impaired *ex vivo* IFN-γ production indicated that recombinant IFN-γ can be a safe and effective adjuvant therapy to augment *in vivo* fungal immunity in cases of CGIFRS, as seen in other chronic fungal infections.[9,10]

CGIFS is a rare condition, and, consequently, there is a lack of a management consensus in the literature,[4] although it is generally accepted that any treatment should involve a combination of surgery and antifungal treatment at a minimum. One recent CGIFRS case report proposed a management approach that involves a combination "conservative" surgery and long-term oral antifungals.[11] Our treatment approach for this reported case broadly followed similar lines, but used a more radical surgical approach to resect all macroscopically involved tissue. Furthermore, and based on the immunologic profile elicited, IFN-γ was also administered. This, indeed, to our knowledge, is the first report of the successful use of IFN-γ as an adjuvant therapy in a case of CGIFRS. This not only provides another management option in patients with CGIFRS but may in fact reduce the need for radical surgical procedures in the future. It is likely, however, that the treatment strategies in CGIFRS will continue to evolve as the immunopathologic mechanisms that underlie this disease are better defined.

We acknowledge that this study's findings are based on a single patient, but the prospect of generating a case series is highly unlikely in view of the rare nature of this condition. Nevertheless, further research is clearly needed to validate these results.

CONCLUSION

This study reports the first *Alternaria*-related case of CGIFRS. Our findings suggest that CGIFRS may be associated with an abnormally exaggerated host Th17 response causing failure to clear the fungal pathogen with refractory fungal infection of mucosal membranes, resulting in chronic tissue inflammation. This study sheds new light on the potential immunologic mechanisms that underlie this disease and also highlight a potential role for IFN-γ as an adjunctive therapeutic option.

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