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Intravenous Immunoglobulin Treatment for Macrophage Activation Syndrome Complicating Chronic Granulomatous Disease

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Abstract
Objectives Chronic granulomatous disease is a rare phagocyte disorder characterized by an increased susceptibility to infections and inflammatory complications. We describe two patients with chronic granulomatous disease (CGD) complicated by macrophage activation syndrome (MAS) (secondary hemophagocytic lymphohistiocytosis) treated with intravenous immunoglobulin (IVIG).
Methods A report of two cases of CGD complicated by MAS who were successfully treated with IVIG was made, and a comparison was made with ten other cases reported in the literature.
Results MAS is a severe potentially fatal complication of CGD. Most cases are associated with *Burkholderia cepacia* and leishmaniasis infection. The treatment of these patients varies between centers, and one example is the use of the HLH-2004 protocol. IVIG could be an effective first line option for this complication in CGD patients.
Conclusions The exaggerated inflammatory response characteristic of CGD patients could play a role in the development of this complication. IVIG appears to be a safe and effective first line treatment in these patients.

Keywords Chronic granulomatous disease · hemophagocytic lymphohistiocytosis · macrophage activation syndrome · intravenous immunoglobulin

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Introduction

CGD is a phagocytosis deficiency produced by mutations in the genes that encode the subunits of the nicotinamide–adenine dinucleotide–phosphate oxidase enzyme complex (NADPH oxidase), most cases have an X-linked inheritance (65–70%), and the rest have an autosomal recessive (AR) pattern [1].

The NADPH oxidase enzyme is responsible for superoxide production in activated phagocytes as a major pathogen killing mechanism (respiratory burst). In CGD, NADPH oxidase is a non-functional enzyme; therefore, these patients have an increased susceptibility to infections by staphylococci, fungi and certain gram negative bacteria including *Burkholderia cepacia* [2]. Another relevant aspect of the disease is the tendency to develop inflammatory complications such as inflammatory bowel disease, granuloma development or inflammatory lung diseases [3].

Macrophage activation syndrome (MAS) is a severe and life-threatening complication seen in patients with autoimmune diseases. MAS shares most of the clinical features of hemophagocytic lymphohistiocytosis (HLH) [4]. Some authors consider MAS as a secondary form of HLH [4]. MAS is characterized by proliferation of macrophages and T-lymphocytes, resulting in an exaggerated inflammatory response, characterized by continuous fever, purpura, hepatosplenomegaly, mental status alterations, coagulation disorders, hypofibrinogenemia and increased erythrocyte sedimentation rate [5]. The presence of hemophagocytosis in the bone marrow, cerebrospinal fluid or spleen is considered as the key feature of this syndrome, although many severe MAS cases have been described without overt bone marrow hemophagocytosis [5]. It is known that CGD monocytes produce large amounts of pro-inflammatory

cytokines, and such phenomenon could be partially responsible for the development of MAS/HLH. Recently, there have been increasing reports of MAS/HLH in patients with CGD [6–11].

Intravenous immunoglobulin (IVIG) is used to treat patients with primary immunodeficiencies but not routinely used in CGD [12]. IVIG has emerged as an important immunomodulatory medication at high doses to treat a wide range of autoimmune and inflammatory disorders [12]. IVIG has been shown to effectively decrease MAS [13]. We report two CGD patients with associated infection and MAS who had clinical improvement with IVIG treatment.

Methods

Two cases of CGD patients complicated with MAS are reported. A Pubmed search was performed using the keywords: chronic granulomatous disease, hemophagocytic syndrome, hemophagocytic lymphohistiocytosis and intravenous immunoglobulin. A qualitative analysis was performed, and the data were summarized, emphasizing clinical features, microorganisms involved and type of treatment.

Case 1

A 3-year-old male diagnosed with CGD secondary to a *CYBB* gene mutation was admitted to our hospital in January 2008 with a history of low-grade fever (38.5°C), diffuse abdominal pain and bloody diarrhea. He had a previous history of BCG-itis and severe recurrent infections (lymphadenitis, abscesses, septic arthritis and pneumonia) and was receiving prophylactic treatment with trimethoprim–sulfamethoxazole, itraconazole and interferon-gamma.

On admission, his vital signs were heart rate of 136 bpm, respiratory rate of 32 bpm, temperature of 39°C and blood pressure of 110/60 mmHg. Physical exam was unremarkable. Laboratory tests revealed hemoglobin of 10.4 g/dl, leukocytes of 4,100/mm3, absolute neutrophil count of 2,100/mm3, total lymphocytes of 1,600/mm3, platelet count of 399,000/mm3 and C reactive protein of 4.83 mg/l. Patient was started on i.v. ceftriaxone, with improvement of gastrointestinal symptoms, but developed persistent high-grade fever and toxic appearance. On the seventh day of admission, blood culture was reported positive for gram-negative rods, and i.v. ciprofloxacin was added to the antibiotic regimen. *Burkholderia cepacia* was identified and was sensitive to ciprofloxacin. Patient developed septic shock on the eighth day of hospitalization and was transferred to the intensive care unit. The possibility of MAS was considered based on the presence of splenomegaly, thrombocytopenia, transaminitis, increase in ferritin level and coagulopathy with elevated D-dimer. Patient received i.v. dexamethasone (8 mg/day) and high-dose IVIG (2 g/kg/day, single continuous infusion) (Table I). Patient had clinical improvement and was discharged after 32 days of hospitalization, with normalization of liver function test, triglycerides and blood count. He has been stable for 3 years, with no significant medical problems.

Case 2

A 5-year-old male with history of BCG vaccination at birth complicated with persistent cervical lymphadenitis, with NBT and DHR tests compatible with diagnosis of CGD, was our second case. He was on trimethoprim–sulfamethoxazole, itraconazole and interferon-gamma prophylactic treatment. The patient was admitted with severe pneumonia in April 2010. Meropenem, vancomycin, amphotericin-B and anti-TB drugs were started without significant clinical improvement. Two weeks after admission, the patient had persistent fever, hepatomegaly associated with thrombocytopenia, high ferritin and transaminases levels, hypertriglyceridemia and elevated D-dimer levels. Patient was diagnosed with MAS and received IVIG (1 g/kg/day as a single continuous infusion). Blood cultures were positive for *Pseudomonas stutzeri*. Thrombocytopenia resolved immediately after administration of IVIG, and clinical condition improved (Table I). Normalization of laboratory tests was seen over a period of 2 weeks. The patient has remained stable for more than a year.

Results

With these two current cases, a total of 12 CGD cases with associated MAS/HLH have been reported. Four of them were associated with *B. cepacia* infections [6–8] and three with visceral leishmaniasis in endemic zones [10]. The characteristics of the patients with CGD and MAS/HLH, including our cases, are shown in Table II.

Half of the patients have X-linked CGD, with four males with no reported inheritance pattern and two females with AR-CGD.

All patients presented with fever, hepatomegaly, thrombocytopenia, anemia, hypertriglyceridemia, high ferritin levels, coagulation disorders and increased D-dimers. According to the results from the HLH-2004 study, ferritin concentrations of >500 mcg/l are 80% specific for HLH [14].

Treatment varied widely among reports, ranging from no treatment for MAS to one patient receiving bone marrow transplantation [11].

Two patients died; one of them was treated according to the HLH-2004 protocol [8]. In this particular case, postmortem examination revealed sporadic hemophagocytosis on the liver, spleen and lymph nodes, but a bone marrow

Table I Laboratory parameters before and after IVIG

	Case 1 MAS	5 days after IVIG	Case 2 MAS	5 days after IVIG
Fever (°C)	39	37	38	36
Hb (g/dl)	10.7	13.3	9.2	11.4
Leucocytes/mm^3	15,780	3,400	15,300	8,800
Platelets	12,000	300,000	67,000	143,000
Fibrinogen (100–400 mg/dl)	179	ND	79	231
Total bilirubin (mg/dl)	9.6	1.63	11.9	4.1
Direct bilirubin (mg/dl)	6	0.85	6.9	1.97
AST (5–40 IU/l)	156	40	1,037	54
ALT (5–36 IU/l)	56	29	227	24
LDH	484	256	1,689	496
Triglycerides (nl<150 mg/dl)	395	210	473	381
Ferritin (12–300 ng/ml)	750	ND	1764	ND

IVIG intravenous immunoglobulin, *ND* not determined

aspirate didn't showed hemophagocytosis [8]. Cultures from lungs and spleen grew *Burkholderia cepacia* and *Stenotrophomonas maltophilia* sensitive to ceftazidime, which was administered as part of the treatment [8]. In one of our patients, *Pseudomonas stutzeri* was documented. *Pseudomonas stutzeri* is a gram-negative aerobic bacterium rarely associated with disease and is considered a saprophyte or contaminant [15]. *Pseudomonas stutzeri* has been associated with infection in HIV patients, but to our knowledge, it hasn't been associated with infection in CGD patients [16].

In other patient with a fatal outcome, specific treatment for MAS/HLH was not specified [10].

Important to note is the finding that all patients that received IVIG survived.

Discussion

We report two patients with CGD who presented clinical and laboratory evidence suggestive of MAS.

We prefer the term MAS instead of HLH for the following reasons:

1 The term highlights the hyperinflammatory state of the macrophage.
2 It doesn't overemphasize the requirement of hemophagocytosis for the diagnosis.
3 Treatment is not well defined as in classical HLH; however, the majority of reported cases with CGD and HLH have not been treated according to the HLH-2004 protocol [14].

Table II Chronic granulomatous disease patients complicated with lymphohistiocytic activation syndrome

Author	Sex	Age	Defect	Microorganism	Ferritin level	BMA	Treatment for HLH/MAS	Outcome	IFN-γ
Sirinavin 2004	M	17 months	NS	*Burkholderia cepacia*	NS	Yes, HP	None	Survived	No
Hisano 2007	F	19 years	p22-phox	*Burkholderia cepacia*	Elevated	Yes, HP	Steroids, CsA	Survived	No
Martin 2009	M	18 years	X-linked	Leishmania	Elevated	Yes, HP	None	Deceased	No
Martin 2009	M	15 years	X-linked	Leishmania	NS	Yes, HP	None	Survived	Yes
Martin 2009	F	9 years	*NCF1*	Leishmania	Elevated	Yes, HP	IVIG	Survived	Yes
Van Montfrans 2009	M	3.5 years	X-Linked	*Burkholderia cepacia, Stenotrophomonas maltophilia*	Elevated	Yes, No HP	CsA, DXM, etoposide	Deceased	No
Parekh 2010	M	12 years	NS	NS	Elevated	Yes, HP	IVIG, steroids	Survived	NS
Parekh 2010	M	6 years	NS	*Staphyloccocus epidermidis*, *Enterobacter cloacae*	Elevated	Yes, HP	IVIG, steroids, splenic irradiation	Survived	Yes
Parekh 2010	M	7 years	NS	NS	NS	Yes, HP	IVIG, steroids	Survived	Yes
Araujo 2011	M	10 months	X-linked	Herpesvirus 6, BCG, fungus	Elevated	Yes, HP	Steroids, BMT	Survived	No
Present case 1	M	3 years	X-linked	*Burkholderia cepacia*	Elevated	No	IVIG, steroids	Survived	Yes
Present case 2	M	5 years	X-linked	*Pseudomonas stutzeri*	Elevated	No	IVIG	Survived	Yes

HP hemophagocytosis, *NS* not specified, *DXM* dexamethasone, *IVIG* intravenous immunoglobulin, *CsA* cyclosporin A

4 HLH is considered as an uncontrolled immune response due to defective granule dependent cytotoxic pathway, and in MAS, the cause is unknown.

CGD patients have a defect in the respiratory burst of phagocytes but not in their activation. The cells cannot destroy the bacteria and increase their pro-inflammatory cytokine production such as IL-1, IL-6 and TNF-alfa, which, in addition to the pro-inflammatory milieu produced in sepsis, favors the immunologic imbalance, leading to a massive activation of macrophages and lymphocytes, making the patient prone to develop MAS [17].

An interesting finding that could explain the susceptibility of these patients to develop MAS is that T-lymphocytes express NADPH oxidase in a manner similar to the granulocyte-macrophages lineage. The deficiency of the enzyme diminishes the T-lymphocyte receptor signal, and this phenomenon seems to favor the production of Th1 cytokines in murine models of CGD [18]. Th1 cytokines such as interferon-gamma induce activation of granulocyte-macrophages, which may be related to the development of MAS in CGD. An important theoretical concern is that interferon-gamma can induce or complicate the development of MAS [19]; however, 5 out of 12 patients didn't receive interferon-gamma prior to the presence of MAS. Martin et al. reported an 18-year-old male with HLH associated with leishmania who was receiving interferon-gamma and had a fatal outcome [10]. Both of our patients continued to receive the drug without further clinical deterioration. Experts suggest that patients who develop severe infections should receive interferon-gamma on a continuous basis [20]. We recommend monitoring the inflammatory response and cautiously continuing the administration of interferon-gamma, which helps to control the infectious stimuli, particularly fungi.

Cessation of inflammation requires the activation of specific anti-inflammatory pathways involving reactive oxide species (ROS) derived from the NADPH oxidase in phagocytes. A failure to generate ROS leads to a state of hyperinflammation and excessive cytokine release. Human natural killer cell function appears to be intact in CGD in contrast to familial HLH [21], although in one report, van Montfrans [8] et al. speculate that alterations in the gene encoding perforin could be a genetic factor that contributes to HLH.

The association of MAS with CGD is not common; however, in the context of clinical and laboratory manifestations, MAS should be considered. Clinical and laboratory findings such ascytopenias, splenomegaly, ferritin elevation and coagulopathies should not only be considered as complications of a severe infection. There are some reports of patients diagnosed with sepsis or septic shock in whom hemophagocytosis has been demonstrated, as well as reports of patients with HLH without findings of bone marrow hemophagocytosis [4, 5]. Unfortunately, there are cases with MAS and severe coagulopathy which contraindicate bone marrow aspiration, as in our cases.

It is important to initiate early MAS specific treatment, but considering the risk of using high doses of immunosuppressant drugs in a CGD patient, such as those suggested by the HLH-2004 protocol, IVIG appears to be an effective first line therapeutic tool [9] because of its powerful immunomodulatory effect and safety profile [12]. In fact, in six out of the eight CGD patients that survived, MAS was treated with IVIG (Table I). High doses of IVIG have been used, from 1 to 3 g/kg/dose [9, 10].

Another important consideration is that, contrary to the familial form of HLH, generally, MAS/HLH in CGD is not a perpetuating, chronic, relapsing disease because there is no evident cytotoxic defect, although in one case reported by Araujo et al [11]., BMT was necessary. Corticosteroids typically have been used in gastrointestinal and obstructive complications. However, corticosteroids have also been used in addition to antimicrobials to treat refractory infections in CGD, and they are able to suppress inflammation in MAS [22, 23]. It is important to note that microbiologic diagnosis is essential to rule out antibiotic failure [23]. This aspect is important because corticosteroids and immunosuppressants can exacerbate infection if the patient is without optimal antimicrobial coverage [22, 23]. On the other hand, experts suggest that there has to be a balance between halting progressing microbial infection and controlling damaging inflammation in CGD [20]. Corticosteroids were administered to our patient in the first case but were not required in the second. Cyclosporin (CsA) is part of the medications recommended for MAS/HLH. CsA was used in two patients, and only one survived [6, 8]. Low-dose CsA treatment promotes regulatory T-cell induction and expansion. Additionally, calcineurin inhibitors have an antifungal activity, a mechanism desired in refractory fungal infections [24, 25].

Finally, we consider that the presence of MAS should be suspected in CGD patients with a severe infection. Treatment with IVIG appears to be an effective first line treatment to control the exacerbated inflammatory response observed in CGD patients.

Conclusions

MAS is a potential life-threatening complication of CGD patients frequently associated with severe infectious events due to *B. cepacia* and visceral leishmaniasis. The defects of NADPH oxidase favor a hyperinflammatory response with hypercytokinemia and, in severe cases, hemophagocytosis.

In a severely infected CGD patient, MAS should be suspected and investigated in order to initiate an appropriate

treatment. Treatment with IVIG as a first line treatment appears to be effective.

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