

Letter to the Editor

TNF- α blockade in chronic granulomatous disease—induced hyperinflammation: Patient analysis and murine model

To the Editor:

Chronic granulomatous disease (CGD), a genetic deficiency in the phagocyte nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2), leads to severe recurrent infections but also to exuberant inflammatory responses. Because infections have a major effect on mortality, they have been the main focus of CGD research and therapies, resulting in markedly increased survival. Because of the improved management of infections, inflammatory complications are now an increasingly important problem. Almost any organ can be affected, with the gut being probably the most common site.^{1,2} Although hyperinflammation might not lead to a major increase in mortality, it is associated with high morbidity.

A breakthrough in research on CGD-induced hyperinflammation was the generation of Nox2-deficient mice with CGD, leading to the development of a skin model of inflammatory complications by Dinarello.⁴ Indeed, injection of sterile fungal cell wall and more specifically β -glucan into the skin of mice with CGD leads to massive hyperinflammation and ultimately granuloma formation.^{4,5} Note that injection of sterile bacterial cell wall components did not lead to hyperinflammation.⁵ Underlying mechanisms are still poorly understood; however, a common observation is an increase in levels of proinflammatory cytokines, particularly TNF- α ,^{6,7} which is often cited as a possible culprit in CGD-induced inflammatory states.³

The following lines of argument suggest that TNF- α inhibition might be a pertinent treatment approach: (1) inflammatory cells from patients with CGD release increased amounts of proinflammatory cytokines, particularly TNF- α ; (2) anti-TNF- α treatments have been successfully used in other types of inflammatory diseases (eg, rheumatoid arthritis and Crohn disease); and (3) inflammatory complications in the context of other immunodeficiencies are improved by TNF- α blockers. However, it is not clear whether the increased secretion of TNF- α by leukocytes from patients with CGD is a causative mechanism in hyperinflammation. Yet despite the lack of information about the role of TNF- α in CGD-induced hyperinflammation, there is an increasing off-label use of anti-TNF- α treatments in patients with CGD. Indeed, the use of these compounds in the treatment of CGD-induced inflammatory complications has been suggested in several publications and is included in recent algorithms of CGD management. In fact, short-term treatment with infliximab has been proposed as the second-line treatment in patients with steroid-refractory chronic granulomatous colitis.⁸

We first performed a literature review on the treatment of CGD-induced inflammatory complications with TNF- α inhibitors (see Table E1 in this article's Online Repository at www.jacionline.org). We found indications for off-label use of TNF- α inhibitors in patients with CGD; indeed, we could identify a total of 17 published cases. Patients with autosomal recessive mutations are overrepresented in the collection (11/17 [65%]), and 7 of these presented with inflammatory bowel disease or arthritis as initial symptom (see patients marked by asterisks in Table E1). Note that in general autosomal recessive mutations represent

approximately 30% of patients with CGD. Only in 5 patients was a clear and sustained response to treatment observed. The treatment response seemed genotype dependent: 4 (36%) of 11 autosomal recessive patients responded, whereas only 1 (17%) of 6 X-linked patients appeared to respond. Because most patients received concomitant treatment with other compounds, their response to treatment could not unequivocally be attributed to the TNF- α inhibitors. In the context of other indications (eg, inflammatory bowel disease and rheumatoid arthritis), increased infection risk caused by TNF- α inhibitors has been observed.

Our literature review of treatment in patients with CGD also raises this concern: among the 17 described cases, 7 infections were reported, often severe, as were at least 2 deaths. The documented infectious complications were possibly also genotype dependent: 3 (27%) of 11 autosomal recessive patients but 4 (67%) of 6 X-linked patients showed severe infection after treatment with TNF- α inhibitors. These infectious complications did not require a prolonged exposure to TNF- α inhibitors because they appeared within weeks after initiation of treatment. Thus we conclude that in patients with X-linked CGD, therapeutic responses to anti-TNF- α treatment appeared rare, whereas infectious complications were frequent. The risk/benefit ratio of anti-TNF- α treatment might be more favorable in autosomal recessive patients.

Only larger studies could provide definitive answers to these questions on a clinical level. However, these studies are difficult to perform because of the rarity of the disease, the heterogeneity of the genotypes, and the high frequency of additional clinical problems and treatments. Also, such studies are likely to expose patients with CGD to an increased infection risk. Thus in our opinion proof-of-principle studies in animals demonstrating that inhibition of TNF- α indeed decreases CGD-induced hyperinflammation are needed. For this purpose, we used Nox2-deficient mice, which are a model of X-linked CGD. Sterile hyperinflammation and increased activation of inflammatory cascades, similar to observations made in human patients, have been well documented in these animals.¹ We therefore investigated the role of TNF- α in hyperinflammation in Nox2-deficient mice with CGD.⁵

Wild-type TNF- α -deficient mice, mice with CGD, and TNF- α -deficient mice with CGD (specifically created for this study) were inoculated intradermally in the ear dorsum with β -glucan, as described previously.⁵ Fig 1 shows measurements in supernatants of skin homogenates of TNF- α , as well as soluble TNF- α receptor 2 (sTNFR2; the latter usually increases in parallel with its cytokine ligand). In both mice with CGD and wild-type mice, β -glucan injection induced higher amounts of TNF- α and sTNFR2 compared with PBS injection. Although no major differences between wild-type mice and mice with CGD were observed at early time points, 7 days after β -glucan injection, TNF- α and sTNFR2 reached higher levels in mice with CGD. To distinguish whether this late increase in TNF- α secretion is a witness or a mediator in CGD-induced hyperinflammation, we (1) inhibited TNF- α in mice with CGD by using etanercept (Fig 2, A) and (2) generated TNF- α -deficient mice with CGD (Fig 2, B). The human TNFR2-IgG1 Fc fusion protein etanercept (Enbrel) or vehicle was injected intraperitoneally (30 mg/kg)

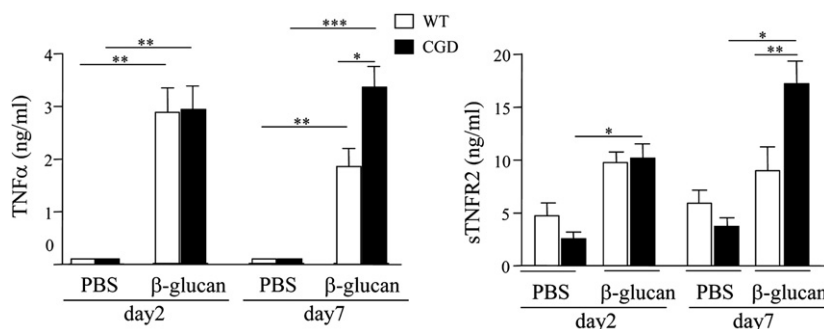


FIG 1. TNF- α overexpression correlates with CGD-induced hyperinflammation *in vivo*. Mice with CGD and wild-type (WT) mice were injected intradermally with β -glucan (200 μ g) or PBS. TNF- α and sTNFR2 production in skin homogenates was evaluated by means of ELISA. All data shown are from 4 to 9 mice and at least 3 independent experiments. Statistical analysis was performed with the unpaired *t* test: **P* < .05, ***P* < .01, ****P* < .001.

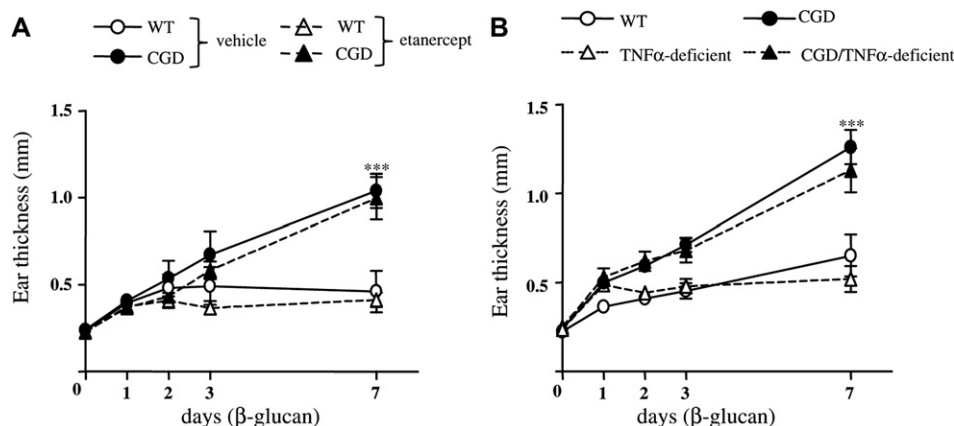


FIG 2. TNF- α is not the mediator of CGD-induced hyperinflammation. Mice were injected intradermally with β -glucan (200 μ g), and ear swelling was measured at the indicated time points. **A**, Mice with CGD and wild-type (WT) mice treated with etanercept or vehicle. **B**, TNF- α -deficient mice, TNF- α -deficient mice with CGD, mice with CGD, and WT mice. All data shown are from 6 to 9 mice and at least 3 independent experiments. Statistical analysis was performed with 2-way ANOVA with the Bonferroni post-test. ****P* < .001 versus wild-type mice treated with vehicle.

1 day before and 2 and 7 days after intradermal injection.⁹ Ear thickness was measured with a digital gauge before and at different time points after intradermal injection. In wild-type mice only mild inflammatory responses were observed, and TNF- α blockade did not have any significant effects (Fig 2, empty circles vs empty triangles; see also Fig E1, A-C, in this article's Online Repository at www.jacionline.org). In mice with CGD, prolonged severe hyperinflammation was observed, as witnessed by ear swelling (Fig 2, black circles). Importantly, neither pharmacologic inhibition nor genetic deletion of TNF- α decreased the extent of inflammation in mice with CGD (Fig 2, B, black triangles). These results were confirmed by means of histologic analysis (see Fig E1).

Our results lead to the following conclusions:

First, genetic deletion or pharmacologic inhibition of TNF- α in mice with CGD mice does not dampen β -glucan-induced hyperinflammation. These data were obtained by using the best available model of CGD-induced hyperinflammation, but 2 limitations should be mentioned: only 1 stimulus (β -glucan) was used, and only 1 organ system (skin) was investigated.

Second, TNF- α blockade is most likely associated with an increased infection risk in patients with X-linked CGD and possibly to a lesser extent in autosomal recessive patients.

Third, the number of clinical cases treated is not sufficient to draw a firm conclusion, but this preliminary analysis suggests that patients with X-linked CGD are less likely to respond to anti-TNF- α treatment than autosomal recessive patients.

Taken together, our results do not allow a definitive conclusion concerning the usefulness of TNF- α inhibitors in patients with CGD. The murine skin model did not yield promising results, but because there is presently no animal model of CGD-induced colitis, this needs to be taken with caution. Also, a murine model of autosomal CGD should be tested because the analysis raises the possibility that autosomal recessive patients with CGD are most likely to benefit from anti-TNF- α treatment.

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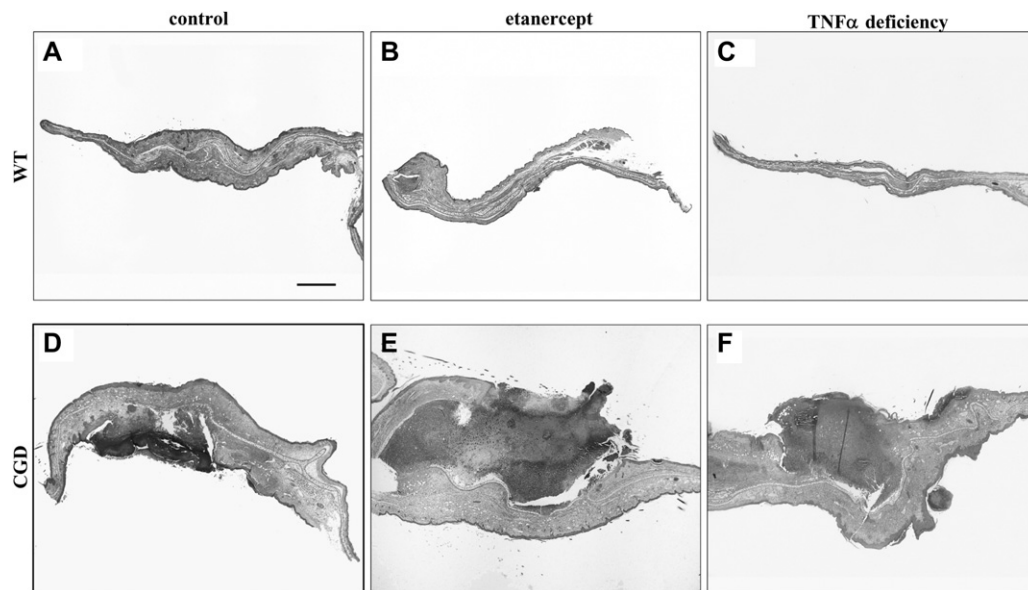


FIG E1. TNF- α blockade does not dampen CGD-induced histologic lesions. Representative pictures of wild-type mice (A-C) and mice with CGD (D-F) 7 days after β -glucan (200 μ g) intradermal injection. TNF- α blockade was obtained by using etanercept injection (Fig E1, B and E) or by means of genetic deletion (Fig E1, C and F). Scale bar = 500 μ m.

TABLE E1. Summary of published cases of patients with CGD treated with TNF- α inhibitors

	No. patients	CGD type	Treatment indication	Anti-TNF- α	Treatment response	Concomitant treatment	Complications	Cultivated germs	Outcome/comment
Marciano, <i>Pediatrics</i> , 2004;114 ^{E1}	1	X-linked	Gastric outlet obstruction	Infliximab	Improvement	Steroids	NS	NS	
Huang, <i>Clinical Gastroenterology and Hepatology</i> , 2004;2 ^{E2}	1	AR (p47 ^{phox})	IBD*	Infliximab	No response	Steroids, sulfasalazin	Aspergillosis (brain, lung)	<i>Aspergillus nidulans</i>	
Wang, <i>Journal of Allergy and Clinical Immunology</i> , 2005;115 ^{E3}	1	X-linked	IBD	Infliximab	Unsustained improvement	Mesalazine, cortisone enemas, 5-aminosalicylic	Tooth abscess, pneumonia, sepsis	Hyphae on lung biopsy, negative culture, <i>Staphylococcus</i> species	
Matute, <i>Blood</i> , 2009;114 ^{E4}	1	AR (p40 ^{phox})	IBD*	Infliximab	No response	NS	NS	NS	Ileostomy
Soncini, <i>British Journal of Haematology</i> , 2009;145 ^{E5}	1	X-linked	Gut GvHD	Infliximab	Questionable or no response	NS	NS	NS	Resolved 6 y after HSCT
De Ravin, <i>Journal of Allergy and Clinical Immunology</i> , 2008;122 ^{E6}	1	AR (p47 ^{phox})	Juvenile idiopathic arthritis*	Etanercept	Resolution		Impaired wound healing	None	Treatment changed
Tommasini, <i>Scandinavian Journal of Gastroenterology</i> , 2010;45 ^{E7}	1	AR (p47 ^{phox})	IBD*	Infliximab, adalimumab	No response	NS	NS	NS	Posttransplantation complicated course, death
Marks, <i>American Journal of Gastroenterology</i> , 2009;104 ^{E8}	2	AR (p47 ^{phox})	IBD	Infliximab	Sustained response	NS	NS	NS	
		AR (p67 ^{phox})	IBD	Infliximab	Questionable or no response	NS	NS	NS	
Freudenberg, <i>Journal of Allergy and Clinical Immunology</i> , 2010;125 ^{E9}	3	AR (p47 ^{phox})	IBD*	Infliximab	Resistant to therapy	Yes but NS	NS	NS	IBD resolved after RIC-HSCT
		AR (p47 ^{phox})	IBD*	Etanercept	Questionable or no response	Yes but NS	NS	NS	Stable on azathioprine, local steroids, pimecrolimus ointments

TABLE E1. (Continued)

	No. patients	CGD type	Treatment indication	Anti-TNF- α	Treatment response	Concomitant treatment	Complications	Cultivated germs	Outcome/comment
		AR (p47 ^{phox})	IBD*	Infliximab, adalimumab	Resistant to therapy	NS	NS	NS	
Uzel, <i>Clinical Infectious Diseases</i> , 2010;51 ^{E10}	5	AR (p47 ^{phox})	Fistulizing IBD	Infliximab	Improvement of colitis, closure of fistula	Prednisone (<0.5 mg/kg/d), aminosalicylic acid, 6-mercaptopurine	Pneumonia	<i>Buckholderia cepacia</i>	
		X-linked	Fistulizing IBD	Infliximab	Questionable closure of fistula, entrapped tract, abscess	High-dose prednisone, 6-mercaptopurine	Entrapped tract, abscess, lymphadenitis	<i>Candida lusitanae</i>	Persistent fistula led to ileostomy and subtotal colectomy
		X-linked	Fistulizing IBD	Infliximab	Unsustained improvement	None	Sepsis, pneumonia, pneumatoses intestinalis	<i>Buckholderia gladioli</i> , <i>Paecilomyces variotii</i> , <i>Pseudomonas aeruginosa</i> ; <i>Acinetobacter baumannii</i> , <i>Aspergillus fumigatus</i> , cytomegalovirus	Total colectomy, distal ileal resection attempted, death
		X-linked	IBD Fistulizing IBD	Adalimumab Infliximab	No improvement Intermittent closure of fistula, with remnant abscess	None Prednisone (1 mg/kg/d), 6-mercaptopurine, mesalazine	None High fever, multiple liver abscesses	Presumed MRSA	Ileostomy
		AR (p22 ^{phox})	Fistulizing IBD	Infliximab	Sustained response	NS	Sepsis (multiple germs)	<i>Buckholderia cepacia</i> , <i>Staphylococcus aureus</i> , <i>Candida krusei</i> , <i>Candida albicans</i> , <i>Aspergillus terreus</i>	Death

All available publications are retrospective studies or case reports of patients having received off-label treatment with TNF- α inhibitors (infliximab, adalimumab, and etanercept). In most cases other anti-inflammatory treatments were associated. *Patients marked by an *asterisk* presented with inflammatory bowel disease or arthritis as the initial symptom. Patients shown in boldface showed an apparent response to anti-TNF- α treatment.

AR, Autosomal recessive; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplant; IBD, inflammatory bowel disease; MRSA, methicillin-resistant *Staphylococcus aureus*; NS, not specified; RIC-HSCT, reduced-intensity conditioning hematopoietic stem cell transplantation.