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Exogenous Pentraxin 3 Restores Antifungal Resistance and Restrains Inflammation in Murine Chronic Granulomatous Disease

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Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by life-threatening bacterial and fungal infections and hyperinflammation. The susceptibility to aspergillosis in experimental CGD (p47phox−/− mice) is associated with the failure to control the inherent inflammatory response to the fungus and to restrict the activation of inflammatory Th17 cells. We assessed whether pentraxin (PTX)3, a member of a family of multimeric pattern-recognition proteins with potent anti-Aspergillus activity, could limit pathogenic inflammation in p47phox−/− mice by curbing the IL–23/Th17 inflammatory axis in response to the fungus. We found that the production of PTX3 was delayed in CGD mice in infection but exogenous administration of PTX3 early in infection restored antifungal resistance and restrained the inflammatory response to the fungus. This occurred through down-regulation of IL-23 production by dendritic cells and epithelial cells which resulted in limited expansion of IL-23R+ γδ+ T cells producing IL-17A and the emergence of Th1/Treg responses with minimum pathology. Thus, PTX3 could be therapeutically used for the exploitation of NADPH-independent mechanism(s) of antifungal immune protection with limited immunopathology in CGD.

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Possible mechanisms involved in CGD hyperinflammation include decreased degradation of phagocytosed material, redox-dependent termination of proinflammatory mediators and/or signaling, as well as redox-dependent cross-talk between phagocytes and lymphocytes. It has been proposed that different inflammatory responses are triggered as a function of the level of reactive oxygen species and have specific characteristics in terms of physiology and pathophysiology. Defective tryptophan catabolism has recently been shown to contribute to the excessive inflammation in CGD mice with aspergillosis. Tryptophan metabolism regulates both antimicrobial resistance and protective tolerance to inflammatory microbial stimuli. We have shown that the enzyme IDO and the other kynurenine pathway enzymes represent not only effector host defense pathways, but also a means of balancing the generation of Th17 and regulatory T cells (Tregs). As superoxide is a cofactor of IDO, the rate-limiting enzyme in tryptophan degradation along the kynurenine pathway, superoxide-induced activation of IDO is a central mechanism by which the optimal balance of antifungal host defense and immune tolerance occurs.

Tregs and IL-17-producing T cells mediate opposing responses in aspergillosis, as clearly shown in CGD mice where the augmentation of IL-17-driven inflammation occurred concomitantly with the reduction of anti-inflammatory Treg cell responses, resulting in excessive inflammation. Neutralization of IL-17A resulted in beneficial effects in mice with aspergillosis, suggesting that limiting Th17 cell activation could be therapeutically exploited to control infection and inflammation in aspergillosis. These data suggest that in addition to attempt to reverse the primary defect of the NADPH oxidase, limiting and resolving the inflammatory response is key to a successful control of the infection and the associated pathology in the condition of NADH deficiency.

Current prophylaxis with trimethoprim-sulfamethoxazole, itraconazole and, in selected cases, additional IFN-γ is efficient in CGD patients, but imperfect. Infectious complications are
typically treated with systemic corticosteroids, which further suppress immunity and have substantial side effects (16). Gene-replacement therapy for patients lacking a suitable stem cell donor is still experimental and faces major obstacles and risks (17, 18). Additional novel therapeutic options are necessary for this rare disorder (19).

In the present study, we evaluated whether pentraxin (PTX)3, a member of a family of multimeric pattern-recognition proteins with potent anti-Aspergillus activity (20), could have beneficial effects in CGD mice with aspergillosis. Considering that PTX3 acts not only as a nonredundant component of the humoral arm of innate immunity but is also involved in tuning inflammation (21) and autoimmunity (22), the interesting hypothesis was that PTX3 could limit pathogenic inflammation in CGD. We found that the production of PTX3 was delayed in CGD mice in infection, such that early administration of PTX3 increased antifungal resistance and restrained the inflammatory response to the fungus.

Materials and Methods

Animals

Eight- to 10-wk-old C57BL6 wild-type (WT) mice were purchased from Charles River Laboratories or The Jackson Laboratory. Breeding pairs of homozygous p47phox−/− mice, raised on C57BL6 background (23), were purchased from Harlan Laboratories and bred under specific-pathogen-free conditions at the breeding facilities of the University of Perugia, Perugia, Italy. p47phox−/− mice recapitulate several of the features of the human disease and are highly susceptible to Aspergillus infection (23–25). Experiments were performed according to the Italian Approved Animal Welfare Assurance A-3143-01.

A. fumigatus, culture conditions, infection, and treatments

The A. fumigatus was obtained from a fatal case of pulmonary aspergillosis at the Infectious Disease Institute of the University of Perugia (9, 12). For infection, mice were anesthetized by i.p. injection of 2.5% avertin (Sigma-Aldrich) before intranasal (i.n.) instillation of a suspension of 2 × 10⁸ resting conidia/20 μl saline for three consecutive days or intratracheal (i.t.) injection of 5 × 10⁶ resting conidia. Quantification of fungal growth in the lungs was done by the colony forming unit (mean ± SE) and chitin assay (micrograms of glucosamine per pair of lungs) (12). For reinfection, mice surviving the primary i.n. infection were similarly reinfected 40 days later with 5 μg/ml PTX3 and extensively washed thereafter. For the conidialidal activity, the percentage of colony forming unit inhibition (mean ± SE), referred to as conididial activity, was determined as described (27). PMN production of ROI was done by quantifying the release of superoxide anion (O₂−) in the culture supernatants through the measure of the superoxide dismutase-inhibitable reduction of cytochrome c (27). The A₅₅₀ was measured in a Microplate Reader BioRad 550 (Bio-Rad) and the results were expressed as nanomoles O₂−/10⁵ cells.

Cytokine assays

Lung homogenates and serum were assessed for human (for exogenous PTX3) or murine PTX3 by using the DuoSet ELISA kit DY1826 or DY2166 (R&D Systems), respectively. The levels of cytokines in pooled lung homogenates and culture supernatants were determined by specific ELISAs (BD Pharmingen and R&D Systems).

RT-PCR and real-time PCR

Total RNA extraction (TRizol; Invitrogen Life Technologies) and synthesis and PCR of cDNA were done as described (12, 14, 29). RT-PCR for PTX3 was done with the 5′-CCG GTG TTG TCG CTG GTG-3′ and 5′-TCC GCA GTA TGA ACA G-3′ primers, corresponding to positions 117–292 of murine PTX3 mRNA (accession number X83601 http://www.ncbi.nlm.nih.gov/nucore/118124324?report = GenBank; for mBD1 with the 5′-GGC ATT CTC ACA AGT CTT GGA CAA AG-3′ and 5′-AGC TCT AAC AAT TGG TGT TCT CAA AAC AAC AG-3′ primers, and for cathelicidin with the 5′-GGGCTGACTATCACTGCGTCTG-3′ and 5′-CACCAGAACCTCTACAGAGTGG-3′ primers. Real-time PCR was performed using the Stratagene Mx3000P QPCR System and SYBR green chemistry (Stratagene). The thermal profile for SYBR green real-time PCR was at 95°C for 10 min, followed by 40 cycles of denaturation for 30 s at 95°C and an annealing/extension step of 30 s at 72°C. Each data point was examined for integrity by analysis of the amplification plot.

Statistical analysis

Data were analyzed by GraphPad Prism 4.03 program (GraphPad Software). The log-rank test was used for paired data analysis of the Kaplan-Meier survival curves. Student’s t test or ANOVA and Bonferroni’s test were used to determine the statistical significance of differences in organ clearance and in vitro assays. Significance was defined as p < 0.05. In vivo groups consisted of 4–6 animals. The data reported were pooled from three to five experiments, unless otherwise specified.

Results

PTX3 mRNA expression and production in mice with aspergillosis

Given the nonredundant role of PTX3 in anti-Aspergillus innate immune response in vivo (20), we assessed whether PTX3 expression and protein production would be different in p47phox−/− or p47phox−/− mice during infection. PTX3 mRNA was not observed at the basal level in either type of mice, but promptly increased after 6 h postinfection in p47phox−/− mice, reaching a peak level expression at 18–24 h, to decline thereafter. In contrast, a delayed expression of PTX3 occurred in p47phox−/− mice, being PTX3 mRNA detected at 48 and 72 h postinfection (Fig. 1A). Consistent

determined by pan-cytokeratin Ab staining. DCs and epithelial cells were exposed to unopsonized Aspergillus conidia at the ratio of 1:1 at 37°C for 18 h in the presence of 5 μg/ml PTX3 before the assessment of mRNA expression.

Flow cytometry

Staining for cell Ag expression was done as described (12). Cells were analyzed with a FACScan flow cytometer (BD Biosciences) equipped with CellQuest software. Before labeling, FcR blocking was performed. Control staining of cells with irrelevant Ab was used to obtain background fluorescence values. Data are expressed as a percentage of positive cells over total cells analyzed.

Antifungal effector activity and ROI production

For fungidal activity, total lung cells or purified neutrophils were incubated with unopsonized conidia at 1:1 ratio at 37°C. The effect of PTX3 on antifungal effector activity of neutrophils was assessed either by pre-exposing the cells to 100 or 10 ng/ml PTX3 for 2 h before the addition of conidia or by exposing cells to conidia pretreated with 5 μg/ml PTX3 and extensively washed thereafter. For the conidialidal activity, the percentage of colony forming unit inhibition (mean ± SE), referred to as conididial activity, was determined as described (27). PMN production of ROI was done by quantifying the release of superoxide anion (O₂−) in the culture supernatants through the measure of the superoxide dismutase-inhibitable reduction of cytochrome c (27). The A₅₅₀ was measured in a Microplate Reader BioRad 550 (Bio-Rad) and the results were expressed as nanomoles O₂−/10⁵ cells.
with the mRNA expression, PTX3 protein production in the lung occurred earlier in p47<sub>phox</sub><sup>−/−</sup> (between 6 to 24 h) than p47<sub>phox</sub><sup>+/−</sup> mice (between 48 to 72 h), the high levels observed in the latter likely due to the abundance of recruited PMNs, known to store PTX3 in the specific granules (30) (Fig. 1B). Both mRNA expression and protein production returned to basal level at 168 h post infection in both types of mice (data not shown). These results indicate that the production of PTX3 is somehow delayed in p47<sub>phox</sub><sup>−/−</sup> mice, in which the elevated PTX3 levels seen late in infection likely reflects the state of hyperinflammation, as suggested (31, 32). Given the crucial role of PTX3 in promoting conidia phagocytosis (20), the defective PTX3 production observed in p47<sub>phox</sub><sup>−/−</sup> mice may prevent the optimal handling of conidia early in infection.

Efficacy of PTX3 in p47<sub>phox</sub><sup>−/−</sup> mice with aspergillosis

To assess whether early supply of exogenous PTX3 would impact on antifungal resistance of p47<sub>phox</sub><sup>−/−</sup> mice, we administered PTX3 daily for 5 days, starting the day of the infection, to p47<sub>phox</sub><sup>+/+</sup> or p47<sub>phox</sub><sup>−/−</sup> mice according to different treatment schedules that include: 1) different types of infection (i.n. vs i.t.), 2) different dosages, and 3) different routes of administration. Mice were monitored for survival, local fungal growth and dissemination, inflammatory cell recruitment, and lung histopathology. PTX3 significantly increased survival and reduced the fungal growth in the lungs of either types of mice infected i.n. (Fig. 1C) and reduced fungal dissemination into the brain (data not shown). The beneficial effect of PTX3 was: 1) dose-dependent, being the
maximum effect observed at the dosage of 1 mg/kg (Fig. 1C) and 2) partially dependent on route of administration, being less efficacious upon local (i.n.) than systemic (i.p.) administration (Fig. 1D). The levels of PTX3 were much higher in the serum (Fig. 1E) but not in the lung (data not shown) of mice treated with PTX3 i.p. than i.n., a finding suggesting that PTX3-elicited systemic effects are important for its antifungal activity in the lung. Interestingly, mice surviving the primary i.n. infection upon treatment with PTX3 also showed increased resistance to re-infection 40 days later (Fig. 1F). PTX3 was not effective when given prophylactically, (a week before the infection, data not shown), which indicates that PTX3 alone did not modify antifungal immune reactivity and that pathogen opsonization is important for its antimicrobial activity.

On assessing the efficacy of PTX3 in mice infected i.t., we found that the beneficial effect was partially reduced, particularly in p47phox−/− mice (Fig. 1G), a finding indicating that PTX3 may target cells of the upper airways.

PTX3 targets PMNs and epithelial cells

To define possible cellular targets underlying the PTX3 activity in infection, we measured the conidiocidal activity in PTX3-treated mice and found that PTX3 treatment increased the antifungal activity of effector lung cells of p47phox−/−, more than WT, mice (Fig. 2A). We have shown that PMNs, more than alveolar macrophages, have defective conidiocidal activity in p47phox−/− mice with aspergillosis (9). PTX3 was able to increase the conidiocidal activity of PMNs from both WT and p47phox−/− mice (Fig. 2B), although unable to activate the respiratory burst of WT PMNs in response to conidia (Fig. 2C).

Considering that PMN recruitment was decreased in the BAL and lungs (Fig. 3, A and B) after PTX3 treatment, this indicates that PTX3 exerts a fine control of quality over PMN function through NADPH oxidase-independent mechanisms.

In an attempt to unravel possible mechanisms of NADPH oxidase-independent activity of PTX3, we assessed whether β-defensins and cathelicidin, known for their role in host defense and
inflammation in the lung (33, 34), were induced by PTX3 in vivo as well as in vitro on epithelial cells, known to be central participants in innate and adaptive immune response as well as mucosal inflammation (35). Interestingly, we found first that the expression of mBD1 and cathelicidin occurred differently in the i.n. vs i.t. infection, being the expression of both higher in the i.n. than the i.t. infection (Fig. 2D). Second, PTX3 increased the mBD1 mRNA expression both in vivo (Fig. 2E) and on epithelial cells in vitro (Fig. 2F), a finding consistent with the ability of PTX3 to significantly promote conidia phagocytosis by these cells (data not shown). The cathelicidin mRNA was also up-regulated by PTX3 in vivo but this effect was not observed on epithelial cells in vitro (data not shown). Although the relative role of mBD1 and cathelicidin in aspergillosis has yet to be defined, the ability of PTX3 to induce, directly or indirectly, their expression suggests that PTX3 may exploit NADPH-independent mechanisms of antifungal resistance in aspergillosis.

PTX3 ameliorates inflammatory pathology

The protective effects of PTX3 in infection included amelioration of lung pathology, as indicated by the extensive reduction of neutrophil and inflammatory cell infiltrates in the lung parenchyma (Fig. 3A) and BAL (Fig. 3B) of infected WT and p47^phox^−/− mice treated with PTX3 i.n. and, even more, i.p. The numbers and extent of granulomas formations were also greatly reduced by PTX3 treatment, as observed by gross pathology (data not shown). These findings are in line with the observation that lung inflammation following Aspergillus infection involves transient inflammatory cell influx into tissues and alveolar spaces, perivascular edema, and partial consolidation. Nevertheless, following fungal clearance, inflammation rapidly resolves, leaving little structural or morphological evidence to indicate that the response occurred. Together, these data indicate that, by promoting conidial phagocytosis, PTX3 may limit cell recruitment and tissue inflammation, a desirable activity in condition of NADPH deficiency.

PTX3 affects cytokine production and Th cell activation in CGD mice with aspergillosis

As the high susceptibility of p47^phox^−/− mice to infection and inflammation was associated with failure to activate protective Th1 and Treg cell responses and the occurrence of inflammatory Th17 cells (9, 14), we evaluated parameters of inflammatory/anti-inflammatory cytokine production and adaptive Th immunity in WT and p47^phox^−/− infected mice upon treatment with PTX3. To this purpose, we evaluated the mRNA expression and actual cytokine production of inflammatory (IL-12p70, IL-23, IL-17A and TNF-α) and anti-inflammatory (IL-10) cytokines in the lungs as well as the presence of specific transcription factors for Th1 (Tbet), Th2 (Gata3), Th17 (Rorc), and Treg (Foxp3) in CD4^+^ T cells purified from TLN. In infection, the levels of IL-12p70, IL-23, IL-17A, and TNF-α (both in terms of mRNA expression and actual cytokine production) were elevated in the lungs of p47^phox^−/−...
mice but were greatly reduced by treatment with PTX3, particularly at the highest dose given i.p. (Fig. 4A). In the case of WT mice, treatment with PTX3 actually increased p35 mRNA expression as well as IL-12p70 and TNF-α productions. These results are not only in line with previous data (20, 26) and the data of Fig. 5 (see below), but also suggest that the opposite effects on p35 mRNA expression seen in p47phox−/− and WT mice are likely dependent on mechanistic differences rather than the stage of infection. Concomitantly, the levels of IL-10 were greatly reduced in p47phox−/− mice upon infection but restored by PTX3 treatment (Fig. 4A).

In terms of adaptive Th immunity, in line with previous findings (9), Tbet/Foxp3 mRNA expressions (indicative of Th1/Treg activation) were increased in TLN CD4+ T cells from WT mice, while Rorc/Gata3 expressions (indicative of Th17/Th2 activation) were increased in p47phox−/− mice. However, administration of PTX3 increased the expression of Tbet/Foxp3 and decreased that of Rorc/Gata3 in p47phox−/− mice, leaving largely unaffected mRNA expression in CD4+ T cells from WT mice (Fig. 4B). Together, these data suggest that PTX3 affects the balance of inflammatory/anti-inflammatory cytokine production in the lungs of p47phox−/− mice with aspergillosis, inhibits the activation of disease-promoting Th17 cells and promotes the development of protective Th1/Treg cells likely accounting for resistance to infection and reinfection.

**PTX3 restricts the expansion of γδ+ CD3+ T lymphocytes in the lungs in CGD mice with aspergillosis**

It has recently been shown that a population of γδTCR+ T+ lymphocytes producing IL-17A is rapidly expanded in the lungs of p47phox−/− mice after A. fumigatus infection and contributes to the generation of the local inflammatory state and failure to restrict fungal growth (9). To evaluate whether treatment with PTX3 would affect the expansion of γδTCR+ cells, largely responsible for the elevated IL-17A production early in infection (9), we performed phenotypic analysis of total lungs cells in WT and p47phox−/− mice after treatment with PTX3. In contrast to WT mice, a population of γδ+ CD3+ T cells was expanded (from 3 to 9%) in the lungs of p47phox−/− mice upon infection, but was reduced (to 2 and 4%) upon treatment with PTX3 at 1 mg/kg/i.p or i.n., respectively (Fig. 5A). Consistent with the histological findings, treatment with PTX3, at the dose of 1 mg/kg/i.p., also concomitantly restricted the expansion of Gr-1+F4–80+ neutrophils, a population of which was 6-fold expanded in the lungs of p47phox−/− mice upon infection. As IL-17A is a potent chemoattractant of neutrophils in the lungs (36), it is likely that the restriction of γδTCR+ cell expansion may have resulted in the reduced neutrophil recruitment. Together, these findings suggest that the protective effect of PTX3 in p47phox−/− mice relies on the concomitant
restriction of γδTCR+ T cell expansion and Gr-1+ neutrophil recruitment.

PTX3 down-regulates IL-23/IL-23R expression in p47phox−/− mice

The inhibition of γδTCR+ cells expansion in the lungs may occur through different mechanisms (9). Because IL-17A expression in the lung is dependent on IL-23 (37), we have evaluated whether down-regulation of IL-23 production by DCs and epithelial cells would be a mechanism by which PTX3 restrict γδTCR+ cells expansion in p47phox−/− mice. Purified cells from the lungs of uninfected mice were exposed in vitro to conidia in the presence of PTX3, n.d., not detectable, i.e., below the detection limit of the assay. *p < 0.05, stimulated vs unstimulated (−) cells. **p < 0.05, PTX3-treated vs untreated cells. Bars are SE. D and E. Cytokine receptor mRNA expression in total lung cells or purified γδCD3+ cells from mice i.n. infected and treated with 1 mg/kg PTX3 i.p. The results shown represent one representative experiment of three.

FIGURE 5. Effect of PTX3 on γδ+ T cell cell recruitment and IL-23 and IL-23R expression in the lungs of infected mice. A, Phenotypic analysis of total lung cells from i.n. infected mice, treated with 1 mg/kg PTX3 i.p or i.n. Numbers referred to percentages of γδ+CD3+ T cells or Gr-1+ F4–80+ neutrophils over total cells analyzed, 1 day after the last PTX3 administration. B and C, Cytokine gene expression (real time PCR) on dendritic or epithelial cells purified from lungs of uninfected mice and stimulated in vitro with conidia in the presence of 5 μg/ml PTX3. n.d., not detectable, i.e., below the detection limit of the assay. *p < 0.05, stimulated vs unstimulated (−) cells. **p < 0.05, PTX3-treated vs untreated cells. Bars are SE.
but not on purified γδTCR⁺ cells, from WT mice and in p47phox⁻/⁻ mice upon PTX3 treatment. Purified γδTCR⁺ cells expressed high levels of IL-17A that were unmodified by PTX3 treatment (Fig. 5E), a finding suggesting that γδTCR⁺ cells are not directly targeted by PTX3. As PTX3 apparently also failed to modulate IL-23R expression on purified γδTCR⁺ cells in vitro (data not shown), these results indicate that cytokine production and cytokine receptors expression are coordinately regulated in infection by PTX3.

**PTX3 increases the therapeutic efficacy of voriconazole**

Current prophylaxis with itraconazole and, in selected cases, additional IFN-γ is efficient, but imperfect in CGD patients (38). A significant recent progress toward new antifungal (e.g., voriconazole and posaconazole) therapy has allowed survival of most patients into adulthood (16). All the above findings prompted us to evaluate whether the immunomodulatory activity of PTX3 could be exploited to increase the therapeutic efficacy of voriconazole in p47phox⁻/⁻ mice with aspergillosis. To this purpose, mice received PTX3 or voriconazole alone at the optimal doses (i.e., at 1 mg/kg i.p. for PTX3 and 1 mg/kg intragastrically for voriconazole) or together at the doses at which none of the agents achieved the maximum therapeutic effect (i.e., at 0.2 mg/kg for PTX3 and 0.5 mg/kg for voriconazole). Mice were monitored for fungal growth in the lungs and brain and activation of protective Th1/Treg cell responses. Each single agent given alone, at the optimal dose, significantly reduced the fungal growth in the lungs and brain and concomitantly decreased TNF-α production and increased IL-10 production (data not shown). At the suboptimal dose, each single treatment reduced to some extent the fungal growth in the lungs and, to a lesser extent, in the brain. However, combination therapy with PTX3 and Voriconazole at suboptimal doses resulted in virtually no detectable fungal growth in the lungs and brain and the occurrence of protective antifungal responses, as judged by the high levels of IFN-γ/Tbet and il110/Foxp3 mRNA expression associated with inhibition of il17a/Rorc (Fig. 6). Therefore, PTX3 appears to work synergistically with voriconazole to restrict the fungal growth, decrease the lung inflammatory response, and activate protective Th1/Treg cell responses.

**Discussion**

The results of our study shows that restraining pathogenic inflammation could be a successful therapeutic strategy in CGD. Excessive inflammation in CGD is not solely the result of unresolved infection, but results from an intrinsic defect in the control of inflammation. One
important corollary of these new findings is whether therapeutic strategies in CGD should target infection or inflammation. The recent discovery that IL-17A-producing T cells crucially contribute to acute and persistent inflammation in CGD mice with aspergillosis (39) offers novel therapeutic targets for this rare disorder. We show in this study that PTX3 could successfully limit Th17-driven inflammation in CGD to the benefit of the occurrence of antifungal protective Th1 responses and anti-inflammatory Tregs.

Much evidence suggests that γδ+ T cells function in an innate manner, although they are arguably the most complex and advanced cellular representatives of the innate immune system (39). Indeed γδ+ T cells are activated in response to stress to the surrounding tissue and perform a number of functions depending upon the location and type of stress that has occurred (40). γδ+ T cells are involved in resolving inflammation in the lung (41, 42) but are also an important source of IL-17A (9, 37), which can exacerbate autoimmune disease (43). It has been suggested that some γδ+ T cells are poised to produce IL-17A in a rapid manner (43). Expansion of IL-17A-producing γδ+ T cells occurred in Mycobacterium bovis bacille Calmette-Guérin-infected lung and was dependent on IL-23 (37). Indeed, naive γδ+ T cells have been shown to produce IL-17A in response to IL-23 alone, a cytokine known to expand pathogenic TH17 cells (44, 45). A similar mechanism is at work in pulmonary aspergillosis as we have recently found that Th17 activation is defective in mice with genetic deficiency or therapeutic ablation of IL-23 (14) and that ablation of γδ+ T cells early in infection, improves resistance to the infection (9). Despite the fact that IL-23 may serve a protective role in condition of Th1 deficiency (14, 46) and IL-17A is important for an optimal Th1 response (37), it appears that a tight control of the IL-23/IL-23R axis is needed to control innate and adaptive antifungal response in CGD mice. In this regard, it is of interest that PTX3 exert such a control over the IL-23 and IL-23R expression in the lungs of CGD-infected mice. IL-23 expression was indeed profoundly down-regulated in both DCs and epithelial cells, the latter cells known to be crucially involved in the cross-talk with γδ+ T cells (35, 47). Concomitantly, IL-23R expression was decreased in lung γδTCR+ cells upon PTX3 treatment, a finding pointing to the harmonious regulation of the IL-23/IL-23R axis by PTX3.

One interesting observation of the present study is that PTX3 was directly able to target epithelial cells for innate antifungal resistance. Not only was the phagocytic ability of purified murine and human epithelial cells increased by PTX3, but the expression of epithelial defensins was also promoted by PTX3. In response to infection, defensins are produced within minutes to hours by neutrophils and specialized epithelial cells (48). Some defensins act as chemotaxant for immature DCs (49), thus acting as a potent immunological adjuvant that provides a link between innate and adaptive immunity. Interestingly, the antimicrobial activity of defensins is synergistic with lactoferrin and lysozyme (50, 51). That neutrophil employs both nonoxidative (lactoferrin and proteases) and oxidative (hydrogen peroxide) defense mechanisms against Aspergillus has already been reported (3, 52, 53). We have found that lung mBD1 and cathelicidin are both induced by PTX3 in vivo and, for mBD1, also in vitro from epithelial cells. Given that the expression of innate defensins was higher in i.n. than i.t. infection, this may explain the decreased efficacy of PTX3 in mice infected i.t. Ultimately, an action of PTX3 promptly produced in infection on cells of the upper airways is an expected event, considering that PTX3 binds to and opsonizes conidia for phagocytosis, an activity lost upon conidial germination, micelial growth, and progressive infection (9). Thus, the timing of PTX3 production appears to be crucial. This may explain the relative inability of CGD mice to restrict the initial fungal growth and the rescue by exogenous PTX3 administered early in infection. Finally, the higher levels of defensins induced upon PTX3 administered i.p. than i.n. indicate that PTX3 could set the lung for antifungal defense by remote control, the nature of which is presently under investigation.

The action of PTX3 in DCs is also of interest. In fact, PTX3 differentially conditioned the activity of lung DCs from WT or CGD mice, potentiating the production of IL-12p70 in WT DCs but curbing that of IL-23 in DCs from CGD mice. This not only confirms previous data on the ability of PTX3 to activate DCs for antifungal Th1 responses (20), but also suggests that NADPH oxidase may condition the DCs response to PTX3. That NADPH oxidase is a regulator of DC activity has already been reported (6, 54–56) as well as the molecular mechanisms leading to the differential production of IL-12/IL-23 in DCs (57, 58). How PTX3 differentially condition DCs from WT and CGD mice is not known. In this regard, it is of interest that phagocytosis of apoptotic cells curbs IL-23 release by DCs (59) and that PTX3 regulates the clearance of apoptotic cells by DCs (60). Irrespective of the mechanism, the ability of PTX3 to condition DC activity from CGD mice further suggests that PTX3 may have NADPH oxidase-independent activity. As a matter of fact, PTX3 did not activate the respiratory burst of neutrophils, while potentiating their antifungal effector activity. The same reasoning would apply for epithelial cells, whose immune regulatory activity beyond the antimicrobial function has recently been proposed (6, 35).

One interesting finding of the present study was that PTX3 worked synergistically with voriconazole to restrict the fungal growth and decrease the lung inflammatory pathology. Despite the fact that the routine use of IFN-γ and itraconazole have reduced the incidence of infections in CGD (3), fungal infections have remained a persistent problem in CGD patients. It is desirable that PTX3 will be used in combination antifungal therapy in these patients.

Collectively, this study suggests that PTX3 could be successfully exploited as a novel therapeutic agent with anti-inflammatory properties in CGD and points to the existence of NADPH-independent mechanism(s) of antifungal immune protection that are amenable to manipulation to restore the appropriate control of infection and inflammation in CGD.

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Disclosures
The authors have no financial conflict of interest.

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