IL-10 overexpression predisposes to invasive aspergillosis by suppressing antifungal immunity

To the Editor:

Proinflammatory immune responses are critically required for antimicrobial host defenses; however, excessive inflammation has the potential to damage host tissues thereby paradoxically contributing to the progression of infection. A central negative regulator of inflammatory responses is IL-10, an immunosuppressive cytokine with a wide variety of functions across multiple cell types.[1] Although the role of IL-10 during infection appears to vary for different microorganisms, a largely detrimental role has been attributed to this cytokine during fungal disease.[2] Given the variable risk of infection and its outcome among patients with comparable predisposing factors, susceptibility to invasive aspergillosis (IA) is thought to rely largely on genetic predisposition.[3] The initial investigation of genetic variability at the *IL10* locus led to the identification of single nucleotide polymorphisms (SNPs) influencing its transcriptional activity; thus, IL-10 may be a reasonable candidate for the genetic regulation of susceptibility to IA in high-risk patients.

In a 2-stage, multicenter study involving 413 hematopoietic stem-cell transplantation (HSCT) donor-recipient pairs, we confirmed that SNPs in *IL10* are critical regulators of susceptibility to IA. Using a discovery cohort of donor-recipient pairs (see Table E1 in this article's Online Repository at www.jacionline.org), we analyzed 5 haplotype-tagging SNPs in *IL10* (see Table E2 in this article's Online Repository at www.jacionline.org) and found that the donor, but not recipient, rs1800896 SNP was associated with an increased risk of IA (Fig 1, *A* and see Table E3 in this article's Online Repository at www.jacionline.org). The contribution of the GG genotype to the risk of infection was further illustrated on modeling a recessive mode of inheritance (Fig 1, *B*). In a multivariate model, the donor GG genotype conferred a 2.6-fold increased risk of developing IA after transplantation, and the association test results were further validated in a confirmation case-control study involving patients with similar demographic and clinical characteristics and by a meta-analysis including all enrolled patients (see Table E4 in this article's Online Repository at www.jacionline.org). Furthermore, although no significant differences were observed, the probability of infection-free survival in the discovery set decreased from 88% among patients with the AA genotype to 79% and 75% among subjects carrying the AG or GG genotypes, respectively (Fig 1, *C*).

Using sequence data from the 1000 Genomes Project, we identified all SNPs in linkage disequilibrium (LD) with rs1800896 (see Table E5 in this article's Online Repository at www.jacionline.org), but none of these were exonic. LD around this SNP was limited to the *IL10* locus (see Fig E1 in this article's Online Repository at www.jacionline.org), implying that noncoding variation is likely to drive the association with IA. To ascertain whether rs1800896, or a variant in strong LD with it, influenced gene transcription, we monitored IL-10 mRNA and protein expression in PBMCs from healthy blood donors subjected to *in vitro* infection with *Aspergillus fumigatus*. We observed striking genotype-specific differences, with PBMCs carrying the GG genotype expressing higher transcript and protein levels than those from AA or AG carriers (Fig 2, *A*). A similarly enhanced IL-10 production was observed in monocyte-derived macrophages from GG carriers (Fig 2, *B*), and the overexpression phenotype was independent of specific pattern recognition receptor activation (Fig 2, *C*). Although the ability of macrophages to ingest the conidia remained intact regardless of the genotype (Fig 2, *D*), cells carrying the IL-10 high-producing genotype displayed a 25% decrease in their ability to clear the fungus, as compared to AA carriers (Fig 2, *E*). This defect was dependent on IL-10, because inhibiting IL-10-mediated signals with a neutralizing antibody restored the fungicidal ability. Importantly, the donor GG genotype also differentially regulated the levels of IL-10 in hematological patients, with higher levels present in bronchoalveolar lavages from cases of IA carrying the GG genotype than AA carriers (Fig 2, *F*).

In contrast to IL-10, PBMCs carrying the GG genotype at rs1800896 secreted lower amounts of TNF-α than those from AA or AG carriers after infection (Fig 2, *G*), a finding implying that, under these conditions, GG homozygotes generate lesser inflammatory responses. The dichotomy between IL-10 and TNF-α production according to rs1800896 genotypes was confirmed in human macrophages, in which the same genotype-specific alterations were observed (Fig 2, *H*) and extended to other proinflammatory cytokines such as IL-6, IL-1β, and IL-8 (Fig 2, *I*). Strikingly, we observed that the defect in TNF-α production by macrophages from GG carriers was abolished when IL-10 was neutralized (Fig 2, *J*). In support of this, the addition of IL-10 to cells carrying low-producing genotypes restrained the production of TNF-α in response to infection (Fig 2, *K*). Likewise, the median concentrations of TNF-α were also decreased among hematological patients carrying the IL-10 high-producing genotype (Fig 2, *L*).

We have identified rs1800896 (or a variant in strong LD with it) as the underlying causal variant within the *IL10* locus and disclosed the suppression of immune responses to *A fumigatus* as the primary mechanism explaining the increased susceptibility to infection. The rs1800896 alleles have been shown to physically interact with the transcription repressor poly(adenosine diphosphate–ribose) polymerase 1 and the specificity protein 1 in an

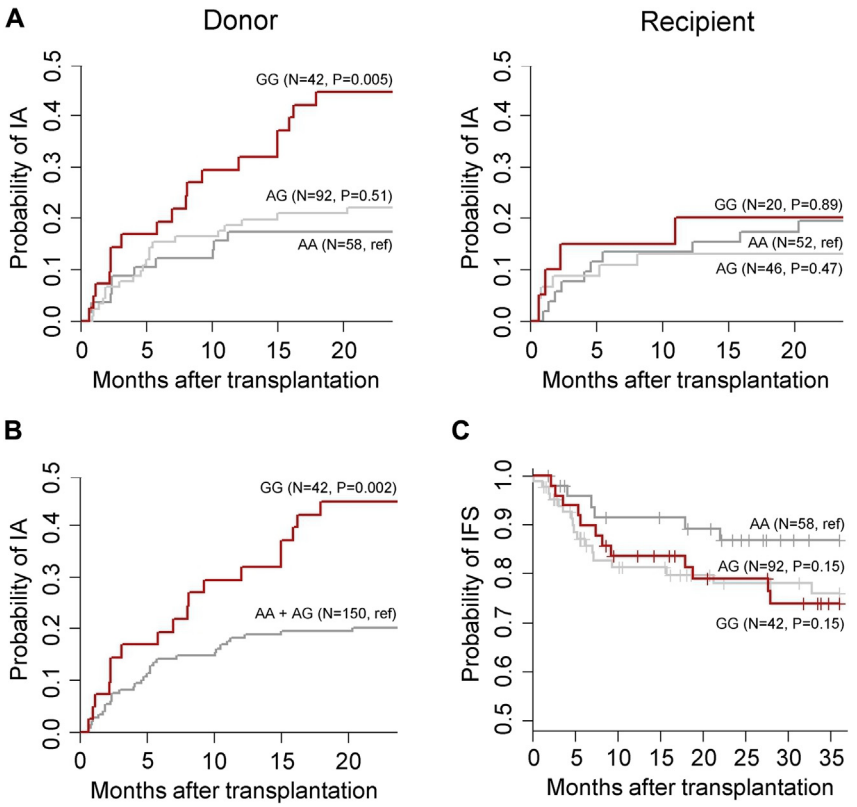

FIG 1. Genetic variation in donor *IL10* increases the risk of IA after HSCT. Shown are the results obtained in a discovery study comprising 216 patients (192 eligible donors and 118 recipients with available patient-level data). Cumulative incidence of IA according to donor or recipient genotypes at rs1800896 **(A)** or a recessive genetic model of donor genotypes at rs1800896 **(B)**. Data were censored at 24 months, and relapse and death were considered competing events. *P* values were calculated using the Gray test. **C,** Infection-free survival (*IFS*) according to donor genotypes at rs1800896. Data were censored at 36 months. *P* values were calculated using the log-rank test.

allele-specific manner (see Fig E2 in this article's Online Repository at www.jacionline.org).[4,5] Because the DNA-binding activity of these transcription regulators varied in different cell types and experimental conditions, additional factors are likely able to regulate expression at this or other sites containing closely linked variants. For example, E26 domain-containing protein Elk1 was shown to bind specifically to alleles at rs3122605 within the *IL10* locus among patients with systemic lupus erythematosus, upregulating circulating IL-10 and correlating with disease activity.[6] Overall, further research is needed to clarify the cell-specific transcription factor(s) activated during IA and that may regulate the rs1800896-dependent IL-10 expression.

The overexpression of IL-10 in macrophages has been reported to promote the autocrine deactivation of these cells, hampering proinflammatory cytokine production and control of pathogen growth.[1] These immunosuppressive effects were confirmed in mouse macrophages to depend on the host's genetic background via the differential production of type I interferons.[7] In humans, a SNP in Forkhead box O3 (*FOXO3*) was found to upregulate IL-10 production, while restraining the inflammatory responses of LPS-stimulated monocytes by regulating TGF-β production,[8] suggesting that several regulatory pathways may occur to explain the immunosuppressive effects of IL-10. In conclusion, our findings may contribute to open new horizons and lay the foundations for risk stratification and preemptive approaches aimed at a more effective management of IA.[9]

We thank Jean-Paul Latgé (Mycology Department, Institut Pasteur, Paris) for generously providing the fungal strain used in this study.

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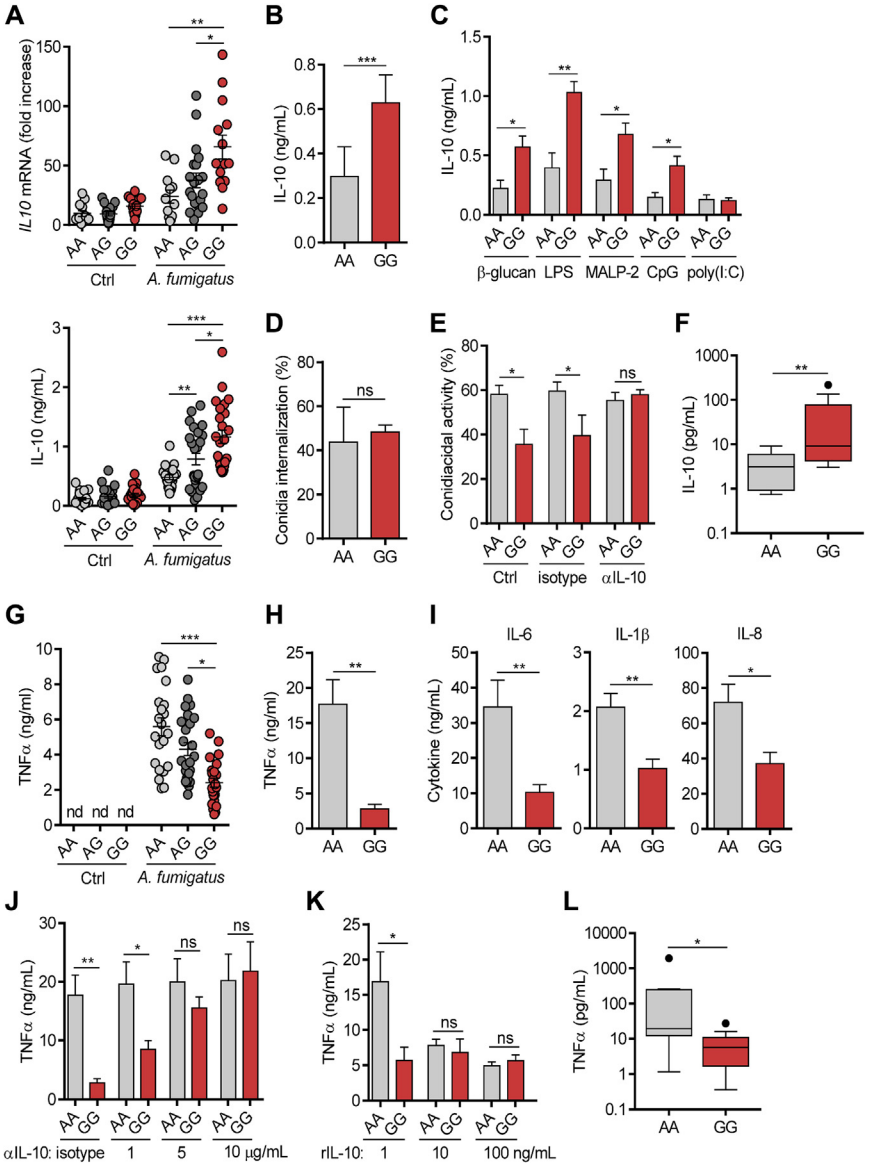

FIG 2. The rs1800896 SNP drives IL-10 overexpression and suppresses proinflammatory cytokine production in response to *A fumigatus*. **A,** Gene expression and secretion of IL-10 by PBMCs carrying different rs1800896 genotypes left untreated (*Ctrl*) or stimulated with *A fumigatus;* n = 12 to 20. IL-10 secretion by macrophages carrying different rs1800896 genotypes stimulated with *A fumigatus* conidia **(B)** or β-glucan, LPS, macrophage-activating lipopeptide-2 (*MALP-2*), 5′-C-phosphate-G-3′ (*CpG*), and poly(I:C) **(C)**; n = 6 to 12. **D,** Phagocytosis efficiency (expressed as percentage of conidia internalization) and conidiacidal activity (expressed as percentage of colony-forming unit inhibition) of macrophages carrying different rs1800896 genotypes stimulated with *A fumigatus* and left untreated *(Ctrl)* or treated with an anti-IL-10 antibody or isotype control **(E)**; n = 4 to 12. **F,** Levels of IL-10 in bronchoalveolar lavage from hematological patients with IA; n = 10 to 12. **G,** TNF-α secretion by PBMCs carrying different rs1800896 genotypes left untreated *(Ctrl)* or stimulated with *A fumigatus;* n = 25. **H,** TNF-α and IL-6, IL-1β, and IL-8 secretion by macrophages carrying different rs1800896 genotypes stimulated with *A fumigatus* conidia **(I)**; n = 6 to 12. TNF-α secretion by macrophages carrying different rs1800896 genotypes stimulated with *A fumigatus* treated with an anti-IL-10 neutralizing antibody or isotype control **(J)**, or recombinant IL-10 **(K)**; n = 6 to 12. **L,** Levels of TNF-α in bronchoalveolar lavage from hematological patients with IA; n = 10 to 12. **P* < .05; ***P* < .01; ****P* < .001. *nd*, Not determined; *ns*, not significant.

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Supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER) (NORTE-01-0145-FEDER-000013), the Fundação para a Ciência e Tecnologia (FCT) (contracts IF/00735/2014 to A.C., IF/01390/2014 to E.T., IF/00021/2014 to R.S., and SFRH/BPD/96176/2013 to C.C.), the Conselho de Reitores das Universidades Portuguesas (CRUP), Portugal (Ações Integradas Luso-Alemãs A-43/16), the Deutscher Akademischer Austauschdienst (DAAD) (project-ID 57212690), the Fondo de Investigaciones Sanitarias (Madrid, Spain) (grant #PI12/02688) and the ERA-NET PathoGenoMics (grant #0315900A). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of potential conflict of interest: K. Lagrou receives grant support from MSD; and received travel support from Gilead and Pfizer. C. Lass-Flörl serves on the board for MSD, Gilead Sciences; serves as a consultant for Gilead, MSD, Pfizer, Schering Plough, Astellas Pharma; receives grant support from Astellas Pharma and Gilead Sciences; receives payment for lectures from Astellas Pharma, MSD, Gilead Sciences, and Pfizer; and receives travel support from Gilead Sciences, MSD, and Pfizer, and Astellas Pharma. O. Kurzai serves as a consultant for Basilea Pharmaceutica Int Ltd; receives payment for lectures from Astellas Pharma GmBH, Basilea Pharmaceutica Int Ltd, and Pfizer Pharma GmbH. J. Sainz receives grant support from TRANSCAN-2; and payments for lectures from MSD and Celgene. J. A. Maertens serves as a consultant for MSD, Basilea, Pfizer, and Gilead; and receives payments for lectures from MSD, Basilea, Pfizer, and Gilead. A. Carvalho receives grant support from FCT (IF/00735/2014) and Northern Portugal Regional Operational Programme (NORTE-01-0145-FEDER-000013); and travel support from Conselho de Reitores das Universidades Portuguesas (CRUP). The rest of the authors declare that they have no relevant conflicts of interest.

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Available online April 4, 2017.
http://dx.doi.org/10.1016/j.jaci.2017.02.034