IL-27 signaling promotes Th1 responses during chronic pulmonary infection with *Aspergillus fumigatus*

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ABSTRACT

Aspergillus fumigatus is the opportunistic fungal pathogen responsible for causing invasive aspergillosis as well as a number of hypersensitivity respiratory disorders. In order to prevent these diseases, *A. fumigatus* must be successfully cleared from the lungs following inhalation. Fungal clearance is associated with Th1 and Th17 responses, while a Th2 response is associated with fungal persistence and the development of allergic disorders. Interestingly, repeated exposure to *A. fumigatus* results in the coevolution of all three of these immune responses in the lungs. For these reasons, it is essential that the host immune system have a way in which to establish and maintain a balance between these immune responses. IL-27 is a heterodimeric cytokine that has been shown to regulate T helper responses. During chronic infection with *A. fumigatus*, IL-27 promotes Th1 responses in the lung by promoting the expression of the T-bet. The absence of IL-27 signaling results in significantly reduced numbers of dendritic cells and exudate macrophages recruited to the lungs. In addition, the diminished Th1 response results in the inability of macrophages to activate to an M1 state. As such, fungal growth and tissue invasion is enhanced in those mice lacking IL-27 signaling. Taken together, this study demonstrates an important role for IL-27 in promoting Th1 responses in the lungs during chronic infection with *A. fumigatus.*

BACKGROUND

Aspergillus fumigatus is a saprophytic fungus found globally, and the most common of its species to cause disease in humans. *A. fumigatus* produce small (2-3 µm) spores known as conidia that are airborne and distributed ubiquitously throughout the environment. On average, individuals inhale upwards of 200 conidia per day[1].Due to their size, conidia are able to avoid cough and mucociliary clearance and enter deeper into the airways. In healthy individuals, these conidia are rapidly cleared by phagocytes such as macrophages and neutrophils, but in the case of immunocompromised individuals, conidia can avoid clearance and germinate into hyphae. This can result in the development invasive aspergillosis, an aggressive fungal disease with an estimated global incidence of 250,000 cases annually, and an associated mortality rate ranging from 30–80%[2]. In addition, repeated exposure to *A. fumigatus* can also result in otherwise healthy individuals developing hypersensitivity respiratory disorders such as allergic bronchopulmonary aspergillosis, farmer's lung and *Aspergillus*-induced asthma. As such, *A. fumigatus* is rapidly becoming a major contributor to the global public health burden.

Figure 1: ***Aspergillus fumigatus***.

Protection against *A. fumigatus* is associated with development of Th1 and Th17 responses, although uncontrolled or prolonged Th17 activation can result in persistent inflammation and tissue damage. A Th2 response on the other hand is associated with fungal persistence and the development of allergic disorders. Interestingly, repeated exposure to *A. fumigatus* results in the coevolution of Th1, Th17 and Th2 responses in the lungs[3]. For these reasons it is essential that the host immune system establish a balance between these responses in order to successfully clear *A. fumigatus*. IL-27 is a regulatory cytokine composed of a p28 subunit and an Epstein-Barr virus Induced Gene 3 (EBI3) subunit that has been shown to either promote or suppress T helper responses depending on the context of the infection[4]. This heterodimeric cytokine is secreted by antigen presenting cells such as macrophages, monocytes and dendritic cells. The p28 subunit is recognized by IL-27Rα (WSX-1), while EBI3 is recognized by gp130. These receptors are commonly found on B cells, NK cells, and especially T cells. IL-27 was originally described as a pro-inflammatory cytokine, promoting Th1 responses through the activation of STAT1 and the Th1-promoting transcription factor T-bet. Later, IL-27 was reported to have anti-inflammatory properties, suppressing the development of Th2 and Th17 cells through the downregulation of the transcription factors Gata3 and RORγt respectively. In addition, IL-27 was reported to suppress the production of IL-2, which may explain its broad suppressive effects on T cells, as well as promote the production of the anti-inflammatory cytokine IL-10. For these reasons, this study sought to examine what, if any, role IL-27 plays in shaping immune responses during chronic infection with *A. fumigatus*.

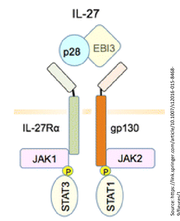

Figure 2: Cartoon of IL-27. Subunits p28 and EBI3, and their respective receptors WSX-1 (IL-27Rα) and gp130.

PROJECT GOAL

To examine the role of IL-27 during chronic pulmonary infection with *Aspergillus fumigatus*.

HYPOTHESIS

One or more regulatory facts, such IL-27, must be in place in order to balance immune responses in the lung chronic *A. fumigatus* infection in order to ensure successful fungal cell clearance.

IL-27 expression is increased following acute infection with *A. fumigatus*.

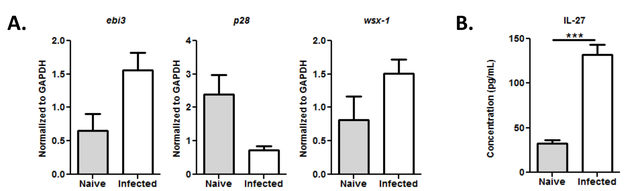

Figure 1: IL-27 expression is increased following acute infection with *A. fumigatus*. Wild type mice were intranasally infected with $5x10^7$ AF293 for 14 days. (A) Gene expression of IL-27 cytokine subunits *ebi3* and *IL-27p28* as well as the IL-27 receptor subunit *wsx-1/IL-27Rα* for naïve WT (n=5) and infected WT (n=8) mice. (B) Protein levels of IL-27 cytokine in the supernatant of lung homogenates from naïve (n=5) and infected WT (n=8) mice as determined by ELISA. Data are expressed as mean ± SEM. All data are from biologically distinct samples. *p<0.05, **p<0.01, ***p <0.001, p values were calculated using Student's t test.

IL-27 is required to suppress *A. fumigatus* invasion into lung tissue

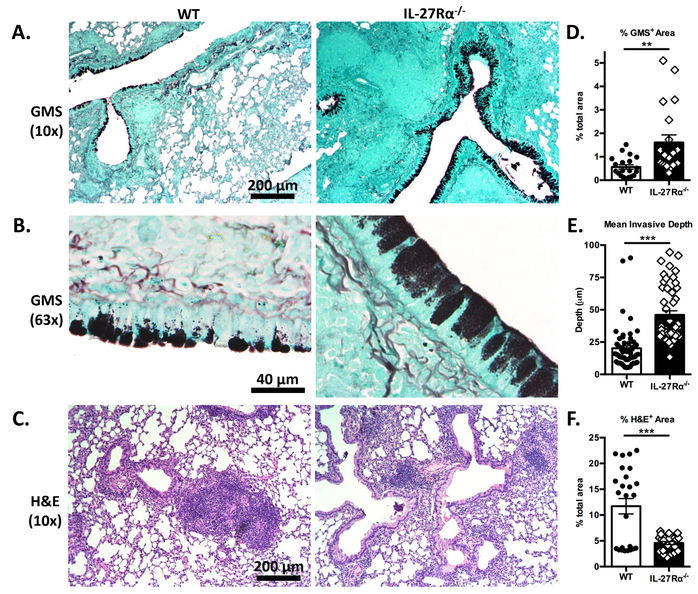

Figure 3: IL-27 is required to suppress *A. fumigatus* invasion into lung tissue. Representative histological images of lung samples from chronically infected WT and IL-27Rα$^{-/-}$ mice that were fixed, sectioned and stained using GMS (A, B) or H&E (C). (A) 10x magnification of WT and KO mouse lungs stained with GMS. (A,B) *Aspergillus* can be visualized as black. (B) 63x magnification of *Aspergillus* invasion into lung tissues. (C)10x magnification of H&E staining: cell infiltrates can be visualized as dark purple. (D) Quantification of % GMS$^+$ area as determined using ImageJ. (E) Quantification of the mean invasive depths from WT and KO mice in µm. (F) Quantification of % H&E$^+$ area as determined using ImageJ. Data are expressed as mean ± SEM. *p<0.05, **p<0.01, ***p <0.001, p values were calculated using Student's t test.

IL-27 signaling promotes T cell activation in the lung during *A. fumigatus* infection

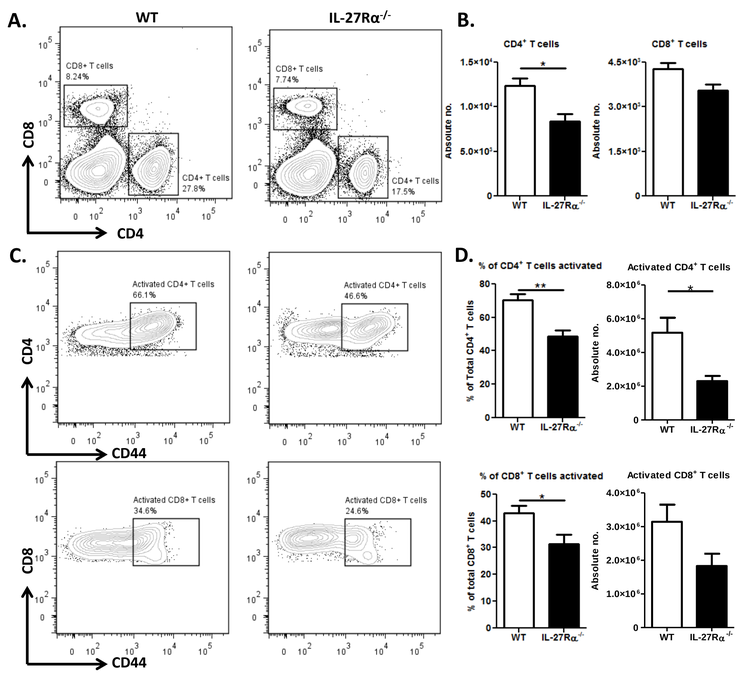

Figure 4: IL-27 signaling promotes T cell activation in the lung during *A. fumigatus* infection. T cell populations in chronically infected WT and IL-27Rα$^{-/-}$ were examined by flow cytometry one day following final instillation of AF293. (A) Representative dot plots of CD4$^+$, CD8$^+$, and (C) activated (CD44$^+$) CD4$^+$ and CD8$^+$ T cell populations. (B) Quantification of CD4$^+$ and CD8$^+$ T cell numbers and (D) percent of CD4$^+$ and CD8$^+$ T cells activated as well as absolute number of activated CD4$^+$ or CD8$^+$ T cells. Data are expressed as mean ± SEM of two independent experiments. All data are from biologically distinct samples. *p<0.05, **p<0.01, ***p <0.001, p values were calculated using Student's t test.

IL-27 signaling promotes Th1 responses in the lung during aspergillosis

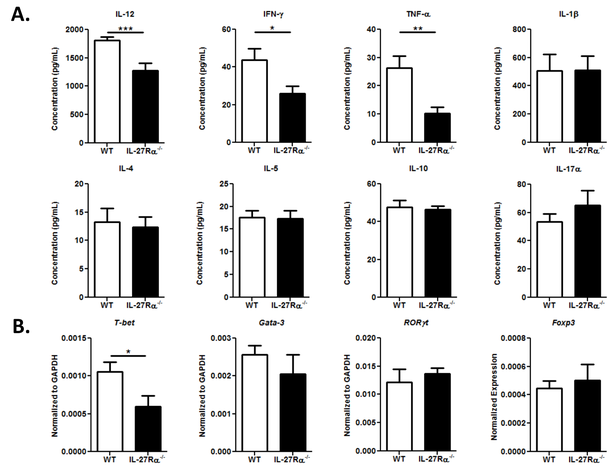

Figure 5: IL-27 signaling promotes Th1 responses in the lung during chronic pulmonary aspergillosis. (A) Th1, Th2 and Th17 cytokine levels in supernatants from lung homogenates of chronically infected WT and IL-27Rα$^{-/-}$ mice as determined by ELISA. (B) Gene expression of Th1, Th2, Th17 and Treg transcription factors determined via qRT-PCR and normalized to GAPDH. Data are expressed as mean ± SEM of two independent experiments. All data are from biologically distinct samples. *p<0.05, **p<0.01, ***p <0.001, p values were calculated using Student's t test.

IL-27 promotes M1 macrophage polarization during *A. fumigatus* infection

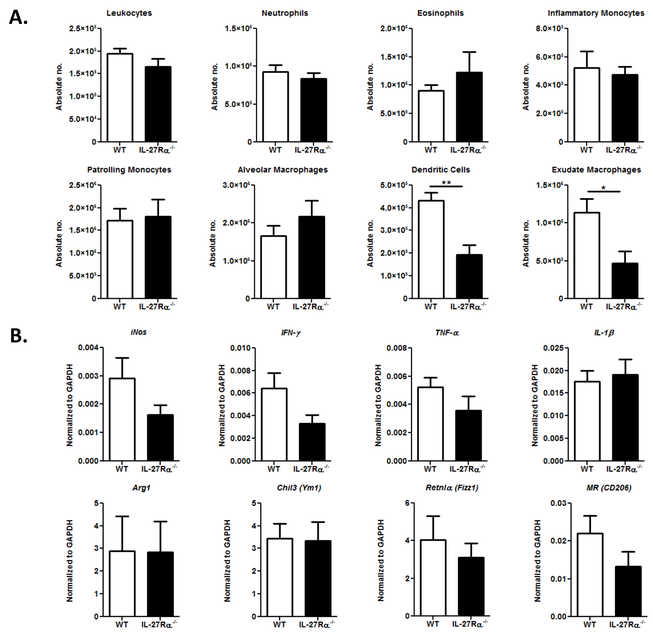

Figure 6: IL-27 promotes M1 macrophage polarization during chronic infection with *A. fumigatus*. Inflammatory cell populations in chronically infected WT and IL-27Rα$^{-/-}$ were examined by flow cytometry one day following final instillation of AF293. (A) Quantification of inflammatory cell populations in WT and IL-27Rα$^{-/-}$ mice. (B) Gene expression of M1 and M2 macrophage markers as determined by qRT-PCR and normalized to GAPDH. Data are expressed as mean ± SEM of two independent experiments. All data are from biologically distinct samples. *p<0.05, **p<0.01, ***p <0.001, p values were calculated using Student's t test.

CONCLUSION

- IL-27 signaling promotes T cell accumulation and activation in the lungs during infection with *A. fumigatus.*
- IL-27 promotes Th1 responses via T-bet expression, which in turn results in M1 macrophage polarization.
- These Type 1 immune responses are required in order to suppress *A. fumigatus* invasion into lung tissues.

REFERENCES

1. DOI: 10.1128/CMR.00055-08
2. DOI: 10.1111/1751-7915.12367
3. DOI: 10.1128/IAI.00508-10
4. DOI: 10.1146/annurev-immunol-032414-112134