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Chapter 4

Pulmonary immune responses against *Aspergillus fumigatus* are characterized by high frequencies of IL-17 producing T-cells

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Abstract

In healthy individuals and in patients with invasive aspergillosis, Aspergillus-specific T-cells in peripheral blood display mainly a Thelper1 phenotype. Although in other fungal infections Thelper17 immunity is important, it was suggested that in aspergillus infection Thelper17 cells do not play a role or may even be detrimental.

Objectives

To compare the cytokine profiles of Aspergillus-specific CD4+ T-cells in peripheral blood and in the lung. To investigate the Thelper phenotype at the primary location of A. fumigatus exposure.

Methods

Lung-derived T-cells and peripheral blood T-cells from COPD-patients were stimulated with overlapping peptides of 6 A. fumigatus proteins. Aspergillus-specific T-cells were identified on the basis of the activation marker CD154 and production of TNFα. In addition, production of the cytokines IFNγ, IL-17, IL-4 and IL-5 by the Aspergillus-specific T-cells was measured.

Results

The majority of lung-derived Aspergillus-specific T-cells displayed a Thelper17 phenotype, and only low percentages of cells produced IFNγ. In contrast, in the peripheral blood of COPD patients Aspergillus-specific T-cells displayed a Thelper1 phenotype, similar as peripheral blood-derived Aspergillus-specific T-cells from healthy individuals.

Conclusions

These data demonstrate that in A. fumigatus infection, similar as in other fungal infections, Thelper17 cells may play a more important role in the immune response than was appreciated until now.
Introduction

*Aspergillus fumigatus* is a mold that can lead to serious infectious complications in immunocompromised patients. Neutropenia and impaired neutrophil granulocyte function are known to be risk factors for invasive aspergillosis \(^1\). In addition, other parts of the innate immune system, like monocytes and macrophages, play an important role in the defense against *Aspergillus fumigatus* \(^2\), and there is mounting evidence that the adaptive immune system, especially T-cells, are also involved in the immune response against *A. fumigatus* \(^3-9\).

Although in fungal infections like candidiasis the role of IL-17 is well established \(^10-12\), in *Aspergillus fumigatus* a role for Thelper17 (Th17) cells was so far not demonstrated. In peripheral blood from healthy individuals \(^4-6\) and from patients with invasive aspergillosis \(^5,7,8\), *Aspergillus*-specific T-cells were detected that primarily displayed a Thelper1 (Th1) phenotype with production of IFNγ. Several papers suggest that Th17 cells do not play a role in the defense against *Aspergillus* \(^13\) or may even have a detrimental effect \(^14\).

However, because it is well known that neutrophil granulocytes are important in the immune defense against *A. fumigatus* and Th17 cells are involved in recruitment, activation and migration of neutrophil granulocytes to the site of a fungal infection, a role for Th17 cells would also be expected in the immune response against *A. fumigatus*. Since the primary location of an aspergillus infection is the lung, we hypothesized that in contrast to *Aspergillus*-specific CD4+ T-cells in peripheral blood, pulmonary *Aspergillus*-specific CD4+ T-cells may have a Th17 phenotype.

In this study, we demonstrate that the frequencies of *Aspergillus*-specific CD4+ T-cells in peripheral blood and lung material are similar, but that the cytokine secretion profiles of these T-cells are different. Peripheral blood derived *Aspergillus*-specific T-cells displayed a Th1 phenotype with production of mainly IFNγ, identical to previous studies on *Aspergillus*-specific T-cell immunity in peripheral blood. However, the majority of lung-derived *Aspergillus*-specific T-cells displayed a Th17 phenotype with primarily production of IL-17, in accordance with the phenotype of Thelper cells pivotal for the protection against other fungal infections.

Material and Methods

Cell collection and preparation

The study was performed according to the Declaration of Helsinki and approved by the local medical ethics committees. After informed consent, peripheral blood samples and pulmonary tissue were obtained from chronic obstructive pulmonary disease (COPD) patients who either underwent a lung transplantation because of COPD Gold stage IV, or a lobectomy because of a peripherally located lung tumor. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Isopaque separation and cryopreserved. Lung-derived mononuclear cells (LMC) were isolated by enzymatic...
digestion from a tissue specimen obtained directly after lobectomy or lung explantation as described previously. Briefly, tissue specimens (1 x 1 cm) were sliced into pieces of 1 mm and incubated for 20 minutes in RPMI with 20mM Hepes, 15% fetal calf serum (FCS), 50 U/ml DNAse type I (Sigma-Aldrich, Zwijndrecht, Netherlands) while shaking at 37°C. Tissue pieces were carefully dried with sterile gauzes and incubated for 60 minutes in medium supplemented with collagenase type I 300 U/ml (Worthington, Lakewood, NJ) while shaking at 37°C. A cell suspension was obtained by grinding the tissue through a flow-through chamber. Mononuclear cells were isolated from the cell suspension by standard density gradient techniques and cryopreserved for later analysis.

**Aspergillus antigens**

Overlapping peptides of the *A. fumigatus* proteins Aspf1, Aspf2, Aspf3, Aspf4, Crf1 and Catalase1, consisting of 15-mer peptides with an 11-amino acid overlap, were synthesized by JPT Peptide Technologies (Berlin, Germany) and dissolved in DMSO.

**Flow cytometry**

PBMC or LMC (0.5x10⁶) were incubated with the combination of overlapping *A. fumigatus* peptide pools (10⁻⁶M) in 96-well plates for 2 hours at room temperature, and then washed to remove DMSO and peptides. Subsequently, the cells were cultured in 150 µl T-cell medium consisting of Iscove’s Modified Dulbecco’s Medium (IMDM, Lonza, Breda, Netherlands), supplemented with 5% fetal calf serum (Gibco, Invitrogen, Bleiswijk, Netherlands), 5% human serum and 100 IU/ml IL-2 (Novartis, Emeryville, CA). After 2 weeks the T-cells were harvested and restimulated with peptide-pulsed or non-pulsed autologous PBMC or LMC (0.5x10⁶). Depending on the expansion of T-cells after this 2-week culture period, cells were split and restimulated with the separate antigens. 2x10⁶ or more cells were split in 7 wells and separately restimulated with overlapping peptides (10⁻⁶M) of the 6 different *A. fumigatus* proteins or with non-pulsed PBMC or LMC. 1x10⁵ cells were split in 3 wells and restimulated with a combination of Aspf1, Aspf2, Aspf3 and Aspf4 in 1 well, a combination of Crf1 and Catalase1 in the 2nd well or with non-pulsed PBMC or LMC in the 3rd well. When the T-cells had minimally expanded (≤0.6x10⁶ cells), cells were split in 2 wells and restimulated with the combination of all proteins or with non-pulsed PBMC or LMC. 1 hour after restimulation 10 µg/ml Brefeldin-A (BFA, Sigma-Aldrich) was added to promote intracellular accumulation of cytokines. 5 hours after restimulation, cells were stained with Pacific Blue-labeled anti-CD4 (BD, Breda, Netherlands), fixated with paraformaldehyde 1% (pharmacy LUMC, Leiden, Netherlands) and permeabilized with saponin 0.1% (Sigma-Aldrich). FITC-labeled anti-CD154 (BD/Pharmingen, Breda, Netherlands), PE-Cy7-labeled anti-TNFα (BD/Pharmingen), APC-labeled anti-IFNγ (BD/Pharmingen), Brilliant Violet 605-labeled anti-IL-17 (IKT/Biolegend, Uithoorn, Netherlands), PerCP-Cy5.5-labeled anti-IL-4 (BD/Pharmingen) and PE-labeled anti-IL-5 (BD/Pharmingen) were added for intracellular staining of the activation marker CD154 and cytokine production. Cells were collected and analyzed on the LSR-II (BD, Breda, Netherlands).
Results

Clinical characteristics of the patients

In this study, we included 10 COPD patients who underwent a lung transplantation or lobectomy, of whom lung material was available for analysis of lung-derived *Aspergillus*-specific T-cells. From 3 of these 10 patients, we also had peripheral blood samples to compare frequencies and cytokine profiles of lung-derived *Aspergillus*-specific T-cells with frequencies and cytokine profiles of peripheral blood derived *Aspergillus*-specific T-cells. Because of limited availability of peripheral blood samples from the included patients with lung material, we included 7 additional COPD patients who underwent a lobectomy, of whom only peripheral blood samples were available for analysis of *Aspergillus*-specific T-cells. Characteristics of included patients are summarized in table 4.1.

All patients had a history of smoking; 12 patients were former smokers, 5 patients were current smokers and tobacco exposure ranged from 25 to 57 pack years. 12 of 17 patients were male. The mean age of included patients was 64 years (range 49-76). Six patients had COPD Gold grade I, 2 patients had COPD Gold grade II and 9 patients had COPD Gold grade IV. There was no clinical or microbiological evidence for aspergillus infection in these patients.

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<th>Surgery</th>
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<td>Lobectomy</td>
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*# n/a not available, pos detectable Aspergillus-specific immune response, neg non-detectable Aspergillus-specific immune response
Aspergillus-specific T-cells with a Th17 profile are present in lung-derived mononuclear cells

Because the primary focus of infection in aspergillosis is located in the lung, we aimed to study the frequencies and cytokine profiles of Aspergillus-specific T-cells in pulmonary material. In previous studies Aspergillus-specific T-cells in peripheral blood could not be detected directly ex vivo, probably due to low frequencies or inability to produce cytokines in the short detection period used in the intracellular staining protocol. We hypothesized that frequencies of Aspergillus-specific T-cells in lung material might be higher than those in peripheral blood and therefore might be detectable directly ex vivo. We stimulated lung-derived mononuclear cells (LMC) with a mixture of overlapping peptides of 6 A. fumigatus proteins and analyzed cytokine production and activation based on the marker CD154 directly ex vivo by FACS. However, in the direct analysis we did not detect Aspergillus-specific T-cells (data not shown).

Therefore, we used a restimulation protocol, in which LMC were stimulated with a combination of overlapping peptides of 6 A. fumigatus proteins, cultured for 2 weeks and subsequently restimulated with peptide-pulsed or non-pulsed autologous LMC. In 8 of 10 analyzed COPD patients we detected Aspergillus-specific T-cells in the lung material based on expression of the activation marker CD154 and production of TNFα after Aspergillus-specific stimulation. In 2 of 10 analyzed COPD patients no Aspergillus-specific T-cells were detected, most likely due to the limited viability of the T-cells in the lung samples. Frequencies of Aspergillus-specific T-cells were calculated as percentage CD4+TNFα+ T-cells after restimulation with autologous LMC pulsed with overlapping peptides minus percentage CD4+TNFα+ T-cells after restimulation with non-pulsed autologous LMC, and ranged from 0.5% to 9.8% of total CD4+ T-cells (figure 4.1A). In 2 patients (C1 and C9) we were able to investigate the specificity of the Aspergillus-specific T-cells by restimulating the T cells after the first Aspergillus-specific stimulation with the separate peptide pools. The results demonstrate that the Aspergillus-specific T-cells in the lungs are directed against a wide range of antigens. In the lungs of patient C1 and C9 CD4+ T-cells were detected against the Aspergillus proteins Aspf2, Crf1 and Catalase1 (figure 4.1B).

Subsequently, we analyzed which cytokines were produced by the lung-derived Aspergillus-specific T-cells. In 4 of 8 patients (C1, C2, C7, C8) with a high frequency of lung-derived Aspergillus-specific T-cells after restimulation with A. fumigatus proteins, ranging between 2% and 9.8% of total CD4+ T cells, we detected Aspergillus-specific T-cells with a Th17 cytokine profile, producing mainly IL-17 and low amounts of IFNγ (figure 4.2A). This Th17 cytokine profile is illustrated in figure 4.2B, in which the cytokine production of the Aspergillus-specific T-cells of patient C2 after restimulation with the overlapping peptides is shown. In patient C1 we were able to analyze the cytokine production after restimulation with the individual Aspergillus proteins. The results demonstrate that the lung-derived Aspergillus-specific T-cells of this patient directed against Aspf2, Crf1, and Catalase all exhibited a Th17 phenotype (figure 4.2C). In 1 of the 8 patients (C9)
Figure 4.1 Aspergillus-specific T-cells in lung-derived mononuclear cells of COPD patients. (A) LMC were stimulated with overlapping peptides of the combination of 6 A. fumigatus antigens and after a 2-week culture period restimulated with the overlapping peptides of the antigens Aspf1, Aspf2, Aspf3, Aspf4, Crf1 and Catalase1. (B) For patient C1 and C9, cells were split in 7 wells and restimulated with the separate antigens or with non-pulsed LMC. Percentages of TNFα producing CD4+ T-cells were calculated as % TNFα+ T-cells of CD4+ T-cells after restimulation with peptide-pulsed LMC minus % TNFα+ T-cells of CD4+ T-cells after restimulation with non-pulsed LMC.

Figure 4.2 Cytokine profiles of lung-derived Aspergillus-specific T-cells. (A) Production of the cytokines IFNγ, IL-17, IL-4 and IL-5 by activated lung-derived CD4+ T-cells after restimulation with overlapping peptides of A. fumigatus antigens depicted as % cytokine-producing T-cells of TNFα+CD4+ T-cells after restimulation with peptide-pulsed LMC. (B) Representative example of FACS-plot of patient C2 after restimulation with unpulsed LMC and after restimulation with LMC pulsed with combination of overlapping peptides of A. fumigatus antigens. CD154+ TNFα+ CD4+ T-cells after peptide-pulsed LMC restimulation were gated and IFNγ, IL-17, IL-4 and IL-5 production of the T-cells is shown. (C) Cytokine profiles of Aspergillus-specific T-cells in patient C1 and C9 after restimulation with the separate A. fumigatus antigens. T-cells were analyzed by FACS after 5 hours of restimulation. Only results of patients in which TNFα expression was induced after stimulation with Aspergillus antigens are shown.
the lung-derived *Aspergillus*-specific T-cells demonstrated a mixed response with production of IL-17, IL-4 and IL-5. By restimulating with the individual *Aspergillus* proteins we were able to demonstrate that the *Aspergillus* specific T-cells directed against Aspf2 primarily produced IL-4 and IL-5, whereas the T-cells directed against Crf1 produced mainly IL-17 (figure 4.2C). In 3 of 8 patients (C3, C4, and C5) with a low number of *Aspergillus*-specific T-cells (0.5-1%) after restimulation in vitro, the lung derived *Aspergillus* specific T-cells mainly produced IFNγ (C3 and C4), or both IFNγ and IL-17 (C5) (figure 4.2A). These results demonstrate that in COPD patients with a strong immune response against *Aspergillus*, the lung-derived *Aspergillus*-specific T-cells mainly exhibited a Th17 phenotype.

*Aspergillus*-specific T-cells in peripheral blood of COPD patients exhibit a Th1 phenotype rather than a Th17 phenotype.

In previous studies that showed no or low frequencies of IL-17 producing *Aspergillus*-specific T-cells in peripheral blood, mainly healthy individuals were analyzed \(^6_{13}\). A repeated analysis in healthy individuals in this study showed identical results with varying frequencies of *Aspergillus*-specific T-cells directed to a broad variety of *A. fumigatus* antigens, displaying a Th1 phenotype (supplemental figure 4.1A-D). In one healthy donor a mixed phenotype was present with production of IFNγ, IL-17 and IL-4 (supplemental figure 4.1B and D).

The analyzed pulmonary samples in the present study were derived from tissue samples from COPD patients who had undergone a lobectomy or lung transplantation. Therefore the high frequencies of IL-17 producing *Aspergillus*-specific T-cells identified in the lung material might be a representation of a generalized Th17 response in COPD patients. To investigate whether *Aspergillus*-specific T-cells in peripheral blood of COPD patients also exhibited a Th17 phenotype like the lung-derived *Aspergillus*-specific T-cells, or whether the *Aspergillus*-specific T-cells exhibited a Th1 phenotype similar to healthy individuals, we analyzed the cytokine profiles of *Aspergillus*-specific T-cells in peripheral blood from COPD patients. From 3 of the 10 patients (C8, C9, C10) of whom we had pulmonary material, we also had peripheral blood samples to directly compare cytokine profiles of *Aspergillus*-specific T-cells in both compartments. In patient C10 no *Aspergillus*-specific T-cells were detected in either lung or peripheral blood after stimulation with *A. fumigatus* antigens most likely due to the impaired viability of the samples (data not shown). In patient C8 and C9 *Aspergillus*-specific T-cells were present in peripheral blood, based on TNFα production (figure 4.3). These *Aspergillus*-specific T-cells were directed against a wide range of *Aspergillus* antigens, as shown in figure 4.3B. *Aspergillus*-specific T-cells in peripheral blood of both patients displayed a Th1 phenotype, mainly producing IFNγ, with low percentages of cells producing IL-17 (figure 4.4A and C), whereas the *Aspergillus*-specific T-cells present in the lung material of these patients mainly exhibited a Th17 phenotype (figure 4.2A and 4.2C).
Figure 4.3 Aspergillus-specific T-cells in peripheral blood of COPD patients. (A) PBMC were stimulated with overlapping peptides of the combination of 6 A. fumigatus antigens and after a 2-week culture period restimulated with the overlapping peptides of the antigens Aspf1, Aspf2, Aspf3, Aspf4, Crf1 and Catalase1. (B) For patient C8 and C9, cells were split in 7 wells and 3 wells respectively, and restimulated with separate antigens or with non-pulsed PBMC. Percentages of TNFα producing CD4+ T-cells were calculated as % TNFα+ T-cells of CD4+ T-cells after restimulation with peptide-pulsed PBMC minus % TNFα+ T-cells of CD4+ T-cells after restimulation with non-pulsed PBMC.

Figure 4.4 Cytokine profiles of peripheral blood-derived Aspergillus-specific T-cells. (A) Production of the cytokines IFNγ, IL-17, IL-4 and IL-5 by activated peripheral blood-derived CD4+ T-cells after restimulation with overlapping peptides of A. fumigatus antigens depicted as % cytokine-producing T-cells of TNFα+CD4+ T-cells after restimulation with peptide-pulsed PBMC. (B) Representative example of FACS-plot of patient C17 after restimulation with unpulsed PBMC and after restimulation with PBMC pulsed with combination of overlapping peptides of Aspergillus antigens. CD154+ TNFα+ CD4+ T-cells restimulation were gated and IFNγ, IL-17, IL-4 and IL-5 production of the T-cells is shown. (C) Cytokine profiles of Aspergillus-specific T-cells in patient C8 and C9 after restimulation with the separate A. fumigatus antigens and with 2 combinations of A. fumigatus antigens, respectively. T-cells were analyzed by FACS after 5 hours of restimulation. Only results of patients in which TNFα expression was induced after stimulation with Aspergillus antigens are shown.
These data indicate that it is most likely the location of the T-cells that determines the phenotype of the Aspergillus-specific CD4+ T-cells and not the type of disease. To verify these data we studied the Thelper phenotype of Aspergillus-specific T-cells in peripheral blood from 7 additional COPD patients (C11-C17). In 6 of 7 additional peripheral blood samples Aspergillus-specific CD4+ T-cells were present after restimulation with overlapping peptides of A. fumigatus proteins (figure 4.3A). Next, we analyzed the cytokine secretion profiles of the peripheral blood-derived Aspergillus-specific CD4+ T-cells. In peripheral blood samples of 5 of 6 COPD patients the Aspergillus-specific T-cells produced primarily IFNγ and only low percentages of the cells produced IL-17 (figure 4.4A). In figure 4.4B the FACS-analysis of peripheral blood-derived Aspergillus-specific T-cells from patient C17 is shown, with IFNγ producing T-cells and lower percentages of IL-4 producing T-cells after restimulation with the overlapping peptides.

Thus, Aspergillus-specific T-cells in these COPD patients showed a Th1 cytokine profile in peripheral blood (figure 4.4A and C), and not a Th17 phenotype like

![Figure 4.5 Differences in cytokine production between Aspergillus-specific T-cells derived from lung and peripheral blood.](image)

(A) Percentages of IL-17- or IFNγ-producing TNFα+CD4+ T-cells after restimulation with A. fumigatus overlapping peptides in lung and peripheral blood. (B) Ratio of IL-17 to IFNγ-producing TNFα+CD4+ T-cells after restimulation with A. fumigatus overlapping peptides in lung and peripheral blood. (C) Percentages of TNFα-producing CD4+ T-cells after restimulation with A. fumigatus overlapping peptides in lung and peripheral blood. * Significant difference in the mean percentage of IL-17 or IFNγ producing TNFα+CD4+ T-cells and in the ratio of IL-17 to IFNγ producing TNFα+CD4+ T-cells after restimulation with A. fumigatus overlapping peptides in lung compared to peripheral blood (p<0.05, two-tailed unpaired Student T test).
in lung material (figure 4.2A and C). The cytokine profiles of Aspergillus-specific T-cells in peripheral blood in COPD patients were similar as in healthy individuals (figure 4.4 and supplemental figure 4.1).

When we compared the percentages of IL-17 and IFNγ producing Aspergillus-specific T-cells in lung with those in peripheral blood from COPD patients we found significant differences (p<0.05), with mainly IL-17 producing T-cells in lung and IFNγ producing T-cells in peripheral blood (figure 4.5A). This is also reflected in the ratio of IL-17 to IFNγ producing T-cells, as depicted in figure 4.5B. In contrast, when we compared percentages of TNFα producing Aspergillus-specific T-cells in lung and peripheral blood, no differences were identified (figure 4.5C), indicating that the percentages of activated T-cells in both compartments were similar.

Discussion

In this study, we have demonstrated the presence of Aspergillus-specific T-cells in the lungs of COPD patients. In the majority of patients, these Aspergillus-specific T-cells exhibited a Th17 phenotype producing besides TNFα primarily IL-17, whereas only low percentages of T-cells produced IFNγ. In contrast, in peripheral blood of COPD patients as well as healthy individuals Aspergillus-specific T-cells showed a Th1 phenotype producing TNFα and IFNγ, and only few peripheral blood-derived Aspergillus-specific T-cells produced IL-17.

In the COPD patients with a strong pulmonary Aspergillus-specific T-cell response (>2% TNFα+ CD4+ T cells), the Aspergillus-specific T-cells primarily exhibited a Th17 phenotype. In contrast in the two patients with a low pulmonary Aspergillus-specific T-cell response (<1% TNFα+ CD4+ T cells) the Aspergillus-specific T-cells mainly produced IFNγ. This may indicate that in those low reactive patients no recent exposure to Aspergillus has occurred.

Previous studies on T-cell mediated immunity against Aspergillus fumigatus investigated the presence of Aspergillus-specific T-cells in peripheral blood from healthy individuals. In these studies low frequencies of Aspergillus-specific T-cells could be detected with a Th1 cytokine secretion profile, producing primarily IFNγ. Isolation of Aspergillus-specific T cells from peripheral blood of healthy individuals by CD154 based selection also demonstrated that the peripheral blood derived Aspergillus-specific T-cells primarily exhibited a Th1 phenotype whereas Candida albicans-specific T-cells selected by the same method exhibited a Th17 phenotype. In addition, analyses of the Thelper phenotype of Aspergillus-specific T-cells in peripheral blood from patients with invasive aspergillosis established no evidence for a role for IL-17 in the immune defense against A. fumigatus. In one study, IL-17 levels in bronchoalveolar lavage fluid from patients diagnosed with invasive aspergillosis were measured and only low levels were found. Some authors suggested that the Th17 pathway even increases susceptibility to aspergillus infections.
However, already for a long time it is known that neutrophil granulocytes are major players in the immune response to fight *Aspergillus* \(^1\), similar as in other fungal infections like *Candida albicans* \(^1\). Because Th17 cells are involved in the recruitment of neutrophil granulocytes to the site of inflammation \(^1\), it is to be expected that Th17 cells have a role in these infections as well. In the host defense against *Candida* infections the role of IL-17 is well established \(^1\): IL-17 deficiency leads to impaired neutrophil recruitment and overt oropharyngeal candidiasis \(^1\).

The results of the present study demonstrating *Aspergillus*-specific T-cells with a Th17 phenotype in lung material from COPD patients, confirm the data by Mirkov et al. on pulmonary aspergillus infections in a study in rats. That study, evaluating the immunological differences at the time of pulmonary aspergillus infection in 2 different immunocompetent rat strains, revealed a difference in IL-17 production. Highest IL-17 levels were identified in the rat strain that demonstrated the fastest clearance of aspergillus infection \(^1\). Our study and the study of Mirkov et al. are in conflict with an earlier human study, in which low levels of IL-17 were measured in bronchoalveolar lavage fluid of immunocompromised patients at the time of invasive aspergillosis \(^1\). However, since this analysis was performed at the time of diagnosis of invasive aspergillosis, an explanation for this discrepancy could be that no *Aspergillus*-specific T-cells were present yet. We and others have demonstrated that at the time of diagnosis no *Aspergillus*-specific T-cells can be detected in peripheral blood, but that *Aspergillus*-specific T-cells are induced in the course of aspergillus infection. Appearance of *Aspergillus*-specific T-cells is correlated with regression of aspergillus lesions \(^7\). \(^8\)

Two studies of T-cell responses in humans \(^2\) and in mice \(^2\) with corneal infections caused by *Aspergillus*, showing induction of both IFNγ and IL-17 producing T-cells at the site of infection, supporting the hypothesis that besides Th1 cells, at the site of infection Th17 cells play an important role, most likely to attract neutrophil granulocytes to the site of aspergillus infection. A recent publication on *Aspergillus* skull base osteomyelitis also implies a role for Th17 in the immune response against *A. fumigatus* and suggests that a defect in the Th17 response in these patients may have caused or contributed to the development of this disease \(^2\). \(^2\)

Whether *Aspergillus*-specific T-cells in the lung and *Aspergillus*-specific T-cells in peripheral blood are separate populations is not clear. Differences in cytokine profiles could point to different effector patterns, that may be caused by different precursor cells, as was previously suggested \(^2\). Alternatively, the IL-17 producing *Aspergillus*-specific T-cells in the lung belong to the resident memory T-cell (T\(_{RM}\)) population, and are of common clonal origin with peripheral blood-derived *Aspergillus*-specific T-cells, as described by Gaide et al.\(^2\). Differences in cytokine profiles between the two populations may be explained by differences in the local milieu, that can influence the inflammatory response, corresponding with an effective response at the site of infection.
In other infections differences in the immune response between peripheral blood and the site of infection have also been described. In influenza infection, influenza-specific T RM have been shown to have a different phenotype compared to T-cells in peripheral blood \(^{25}\), and discrepancy in disease related biomarker signature between peripheral blood samples and pulmonary samples was recently also described for tuberculosis infection \(^{26}\).

In conclusion, this is the first study in humans that investigates the Aspergillus-specific T-cell response in the lung, the site of first exposure to A. fumigatus. Here we demonstrate that Aspergillus-specific T-cells in the lung primarily display a Th17 cytokine secretion profile, whereas Aspergillus-specific T-cells in peripheral blood display a Th1 phenotype. These data indicate that, like in other fungal infections, also in the immunity against A. fumigatus Th17 cells play a role at the site of infection.
Reference List


(13) Chai LY, van de Veerdonk FL,Marijnissen RJ et al. Anti-Aspergillus human host defence relies on type 1 T helper (Th1), rather than type 17 T helper (Th17), cellular immunity. *Immunology* 2010;130:46-54.


Supplemental figure S4.1

Aspergillus-specific T-cells in peripheral blood mononuclear cells from healthy individuals. (A) PBMC were stimulated with overlapping peptides of the combination of 6 A. fumigatus antigens and after a 2-week culture period restimulated with overlapping peptides of the antigens Aspf1, Aspf2, Aspf3, Aspf4, Crf1 and Catalase1. (B) Production of the cytokines IFNγ, IL-17, IL-4 and IL-5 by activated peripheral blood-derived CD4+ T-cells after restimulation with overlapping peptides of A. fumigatus antigens depicted as % cytokine-producing T-cells of TNFα+CD4+ T-cells after restimulation with peptide-pulsed PBMC. (C) For individual H1 and H2, cells were split in 7 wells and restimulated with separate antigens or with non-pulsed PBMC. (D) Cytokine profiles of Aspergillus-specific T-cells in individual H1 and H2 after restimulation with the separate A. fumigatus antigens. Percentages of TNFα producing T-cells were calculated as % TNFα+ T-cells of CD4+ T-cells after restimulation with peptide-pulsed PBMC minus % TNFα+ T-cells of CD4+ T-cells after restimulation with non-pulsed PBMC. T-cells were analyzed by FACS after 5 hours of restimulation.
Aspergillus-specific Th17 cells in lung