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IL-17A is Associated with Alveolar Inflammation and Poor Outcomes in Acute Respiratory Distress Syndrome

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Abstract

Objective—Interleukin-17A (IL-17A) is a pro-inflammatory cytokine known to play a role in host defense and pathologic inflammation in murine models of lung injury. The relationship between IL-17A and inflammation in human lung injury is unknown. Our primary objective was to determine whether IL-17A levels are associated with alveolar measures of inflammation and injury in patients with acute respiratory distress syndrome (ARDS). Our secondary objective was to test whether IL-17A levels are associated with ARDS-related outcomes.

Design, Setting and Patients—We studied two groups of patients with ARDS: 1) patients previously enrolled in a placebo-controlled clinical trial of omega-3 fatty acids performed at five North American medical centers (N=86, ARDS1) and 2) patients with SIRS admitted to an ICU who developed ARDS (N=140, ARDS2). In ARDS1, we used paired serum and bronchoalveolar lavage (BAL) fluid samples obtained within 48 hours of ARDS onset, while in ARDS2, we used plasma obtained within the first 24 hours of ICU admission.

Interventions—None

Measurements and Main Results—We measured circulating IL-17A in ARDS1 and ARDS2. We also measured IL-17A, neutrophil counts, and total protein in bronchoalveolar lavage (BAL) fluid from ARDS1. We found that BAL IL-17A was strongly associated with higher BAL percent neutrophils ($p<0.001$) and BAL total protein ($p<0.01$) in ARDS1. In both ARDS1 and ARDS2, elevated IL-17A was associated with higher SOFA scores ($p<0.05$).

Conclusions—Elevated circulating and alveolar levels of IL-17A are associated with increased percentage of alveolar neutrophils, alveolar permeability and organ dysfunction in ARDS.

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Conflicts of interest All authors have no conflicts of interest to declare.

Keywords

sepsis; cytokine; organ dysfunction; acute lung injury

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a leading cause of acute respiratory failure in the intensive care unit. In spite of progress in the care of patients with ARDS, morbidity and mortality among these patients remains unacceptably high (1). The pathogenesis of ARDS involves a brisk inflammatory response characterized by a predominantly neutrophilic infiltrate and induction of alveolar epithelial and endothelial damage (2). Recruited neutrophils are believed to play a role in ARDS pathogenesis via production of pro-inflammatory cytokines and reactive oxygen species (3, 4). A deeper understanding of the mechanisms of inflammation in ARDS may provide novel therapeutic targets.

Interleukin-17A (IL-17A) plays a key role in neutrophil recruitment in protective and pathologic immune responses (5). IL-17A is the classic effector cytokine of Th17 $CD4^+$ T cells but is also produced by $CD8^+$ T cells (6), $\gamma\delta^+$ T cells (7), innate lymphoid cells (8), neutrophils (9), and NKT cells (10). In murine models of lung infection, IL-17A is critical for the clearance of *Klebsiella pneumoniae* and *Mycoplasma pneumoniae* by promoting chemokine secretion by the respiratory epithelium (11, 12). While IL-17A-mediated responses play a role in pathogen killing via neutrophil recruitment, this may occur at the expense of tissue damage. This “double-edged sword” paradigm was recently shown in a nematode infection model, in which IL-17A was important to parasite killing but contributed to lung damage via neutrophil recruitment (13). In murine models IL-17A has been implicated in lung injury caused by H1N1 influenza infection (14, 15). Thus IL-17A is likely to play a dual role in lung injury, coordinating an early appropriate immune response to pathogens while promoting pathologic inflammation in the lung.

In humans, IL-17A has also been implicated in acute and chronic airway inflammation in diseases where neutrophils play a pathogenic role. In infants with severe bronchiolitis due to respiratory syncytial virus, IL-17A concentrations in tracheal aspirates are correlated with alveolar neutrophilia (16). In asthma, plasma IL-17A associates with disease severity (17) and *IL17A* mRNA levels correlate with sputum neutrophil counts (18). IL-17A has also been implicated in neutrophilic inflammation in cystic fibrosis and COPD (19–22). In ARDS to date, IL-8 has been the main chemokine implicated in neutrophil chemotaxis (23, 24). Taken together, these clinical studies support the notion that IL-17A may participate in neutrophilic inflammation in the human lung.

Given the role of IL-17A in pathologic inflammation in murine models of lung injury and supportive clinical results in other human lung diseases, we sought to determine the relationships between IL-17A levels in the systemic and alveolar space and alveolar inflammation and damage in patients with ARDS. We hypothesized that increasing concentrations of IL-17A would be associated with an increased percentage of alveolar neutrophils and injury and that this process might influence ARDS-related outcomes. We also tested whether these relationships were dependent on IL-8 levels. Since the

pathogenesis of ARDS may differ whether sepsis or trauma is the predisposing condition or if there is direct or indirect lung injury (25–27), we also tested whether the relationship between IL-17A and alveolar inflammation persists in patients with trauma-associated or indirect ARDS.

MATERIALS AND METHODS

Study Populations

ARDS 1: Omega-3 fatty acid trial subjects—Patients meeting criteria for acute lung injury (ALI) as defined by the American-European Consensus Conference in 1994 (28) were enrolled between 2006 to 2008 at five north American centers for a phase II placebo controlled trial of omega-3 fatty acids that did not find a difference between groups (29). All subjects included in this cohort met the more recently determined “Berlin” definition of ARDS (30). This trial was registered in ClinicalTrials.gov as NCT00351533. Written informed consent was obtained from the subject’s legal next of kin and within 48 hours of onset of ARDS, patients were randomized to receive enteral fish oil or 0.9% saline. Bronchoalveolar lavage (BAL) and serum samples were obtained at study entry (day 0) and on days 4±1 and 8±1. On day 0, samples were obtained prior to receiving treatment with enteral fish oil or 0.9% saline. The bronchoalveolar lavage procedure was performed by instilling the same volume of saline into the right middle lobe or lingual and aspirating fluid for analyses (29). Clinical data including Acute Physiology and Chronic Health Evaluation (APACHE) II score, and outcomes such as sequential organ failure assessment (SOFA) score (31), and 28 day mortality were ascertained. Sepsis was defined by the presence of documentation of “sepsis” or “septic-shock” by ICU providers or if the subject met 2 of 4 SIRS criteria and had a positive bacterial culture in the 48 hours prior to study enrollment. Direct lung injury was defined by the presence of an ARDS risk factor of pneumonia, aspiration, drowning, or inhalation.

ARDS 2: Harborview Medical Center systemic inflammatory response subjects—The HMC SIRS cohort has been described previously and were initially enrolled after admission to the ICU by meeting SIRS criteria (32). For this study we limited subjects to those who developed ARDS within 48 hours of ICU admission. We defined ARDS as the presence of bilateral infiltrates on chest x-ray scored by 3 critical care physicians, PaO2/FiO2<300, and absence of clinical evidence of elevated left atrial pressures as a primary cause for the infiltrates. All subjects met the “Berlin” definition of ARDS (30). We used plasma samples obtained within 24 hours of enrollment. In this cohort, sepsis was defined by the presence of 2+ SIRS criteria and the presence of probable or suspected infection as defined by the administration of broad spectrum antibiotics within 3 days of meeting SIRS criteria and continued for at least 5 days or until death or discharge. We defined direct lung injury as subjects with pneumonia or aspiration as an ARDS risk factor. Because this cohort was previously used for genetic studies, subjects were limited to Caucasians. Clinical data including APACHE III score and outcomes including SOFA score and in hospital mortality to day 28 were obtained from the electronic medical record.

All studies were approved by the Division of Human Subjects Research at the University of Washington (Seattle, WA).

Measurements

Cytokine measurements—Circulating (in ARDS1 and ARDS2) and BAL (in ARDS1) concentrations of IL-17A were measured using a chemiluminescent immunoassay (Meso Scale Discovery, Gaithersburg, MD). The lower and upper limits of detection were 0.12 and 5000pg/ml respectively. Plasma and BAL concentrations of IL-8 in ARDS1 were measured by cytometric bead-based immunoassays (R&D Systems, Minneapolis, MN; (29)). Plasma concentrations of IL-8 in ARDS2 were measured using a chemiluminescent immunoassay (Meso Scale Discovery, Gaithersburg, MD).

BAL neutrophil count—We calculated the total cell count of each BAL using Guava ViaCount, a flow cytometric based method (EMD Millipore, Billerica, MA). We used a cytospin preparation of each BAL cell pellet to determine total differential cell populations classified by morphology from which we calculated the percent of neutrophils (29).

BAL total protein—We measured BAL protein concentration using the bicinchoninic acid (BCA) protein assay (Thermo Scientific, Rockford, IL).

Analysis

We identified correlations between $\log_2$-tranformed serum and BAL IL-17A, using a Pearson's correlation test. We then tested for a relationship between IL-17A levels and measures of alveolar inflammation (percent neutrophils) and injury (total protein) in BALF from ARDS1. We used multiple linear regression to test for associations between $\log_2$-transformed IL-17A levels and percent alveolar neutrophils and $\log_{10}$-transformed total alveolar protein concentrations all measured on day 0 (enrollment). We adjusted for age, gender, race, treatment group, presence of sepsis, and APACHE II score. These covariates were selected *a priori* based on known relationships with our endpoint. We then tested whether levels of IL-8, a known chemoattractant for neutrophils in ARDS, attenuated the relationship between IL-17A and alveolar inflammation and injury. In subsequent analyses, we tested for associations between paired BAL IL-17A concentration and percentage of alveolar neutrophils in subjects who survived and had bronchoscopies on days 4 (BAL n=64, serum n=71) and/or day 8 (BAL n=40, serum n=43). We also performed subgroup analyses dividing subjects into groups with trauma-associated or sepsis-associated ARDS and direct or indirect ARDS. We used logistic regression to assess the relationship between IL-17A and sepsis-associated ARDS.

We next tested for relationships between plasma IL-17A levels and clinical outcomes, 28-day mortality and maximum SOFA score over first 7 days, using multiple logistic regression and multiple linear regression respectively. We first tested relationships in ARDS1 with ARDS2 serving as a validation cohort. We adjusted for age, gender, race, treatment group, and presence of sepsis in ARDS1 and age, gender, and presence of sepsis in ARDS2. We did not adjust for severity of illness because we believe that IL-17A may causally participate in organ dysfunction and death in ARDS. We also tested whether inclusion of levels of

circulating IL-8 in the multivariate model attenuated associations between IL-17A levels and outcomes. Analyses were performed using STATA XI (College Station, TX).

RESULTS

Characteristics of subjects in ARDS1 and ARDS2 are shown in Table 1. In ARDS1, 86 subjects had serum and BAL samples available. ARDS1 subjects were predominantly male (63%) and were 50 years of age on average. ARDS2 subjects were also predominantly male (73%), and were 54 years of age on average. In ARDS 1, APACHE II scores were 22 points on average consistent with moderate severity of critical illness (33). In ARDS2, APACHE II scores were 26 points on average. In terms of risks for ARDS, infection (sepsis and/or pneumonia) was the predominant defined clinical risk for ARDS in both cohorts although ARDS1 included patients with trauma while ARDS2 did not. Other sources of infection and categories of pathogens are listed in Supplemental Table 1 (supplemental digital content 1). The average highest SOFA score within 7 days was 8.7 ± 3.6 in ARDS1 and 7.0 ± 3.3 in ARDS2. The two cohorts had similar 28-day mortality: 15% in ARDS1 and 18% in ARDS2.

IL-17A levels associate with the percentage of alveolar neutrophils and total protein

Day 0-1 plasma concentrations of IL-17A were similar in both groups (median (IQR)): ARDS1 (3.5 pg/ml (1.6-9.6)) and ARDS2 (3.7 (1.4-11.5; Table 1). Day 0 BALF concentrations of IL-17A in ARDS1 tended to be lower than plasma values. We found that BAL and serum IL-17A levels acquired simultaneously on study days 0, 4, and 8 were highly positively correlated ($p<0.001$; Supplemental Figure 1A-C; supplemental digital content 1).

We then tested for associations between IL-17A concentrations in the BAL and alveolar inflammation as measured by percentage of alveolar neutrophils. We found that a two-fold increase in BAL IL-17A was strongly associated with increasing percent of alveolar neutrophils on day zero ($p<0.001$; Table 2), day 4 ($p<0.001$), and day 8 ($p<0.001$; Supplemental Table 2; supplemental digital content 1). The relationship persisted after adjustment for age, gender, race, treatment group, sepsis, and APACHE II score on study day 0 ($p<0.001$; Table 2). Because neutrophil mediated endothelial and epithelial injury likely contributes to accumulation of protein-rich edema fluid in the alveoli (34), we tested for associations between IL-17A and alveolar total protein levels. We found that a two-fold increase in BAL IL-17A was associated with increased BAL protein concentrations on day zero ($p=0.007$), day 4 ($p<0.0001$) and day 8 ($p=0.001$) and this relationship persisted after adjustment for the same covariates used above (Supplemental Table 3, supplemental digital content 1).

We next tested for associations between systemic IL-17A concentrations and alveolar inflammation. We found that a two-fold increase in serum IL-17A was associated with increasing percent of alveolar neutrophils on day 0 ($p=0.01$; Table 2), day 4 ($p=0.01$) and day 8 ($p=0.002$; Supplemental Table 2; supplemental digital content 1). This relationship remained after adjusting for age, gender, race, treatment group, sepsis, and APACHE II score on day 0 ($p=0.02$). In contrast to BAL IL-17A, circulating IL-17A levels were not

significantly associated with BALF total protein levels (Supplemental Table 2, supplemental digital content 1).

Prior reports have implicated the chemokine IL-8 as a potential mediator of neutrophilic inflammation in the lung in ARDS (35). In univariate analyses BAL IL-8 concentration was associated with increasing percentage of alveolar neutrophils ($p<0.001$; Table 2). In contrast levels of serum IL-8 were not associated with percentage of alveolar neutrophils. We next tested whether the relationship between IL-17A and alveolar inflammation was independent of IL-8 levels. When we included BAL IL-8 in the multivariate model, BAL IL-17A levels remained strongly associated with the percentage of alveolar neutrophils and explained a large portion of the variance ($p=0.001$; $R^2=0.37$; Table 3).

The pathophysiology of ARDS may differ substantially after direct or indirect lung injury or if trauma or sepsis is the predisposing ARDS risk factor. When we compared circulating levels of IL-17A between subjects with indirect or direct lung injury, there was no difference between groups in ARDS1 or ARDS2. In subgroup analysis in ARDS1 of those with indirect or direct lung injury, elevated BAL IL-17A levels were significantly associated with percentage of alveolar neutrophils in both indirect and direct lung injury (Supplemental Table 4, supplemental digital content 1). However, serum levels of IL-17A were only associated with the percentage of alveolar neutrophils in subjects with direct lung injury. When we compared circulating levels of IL-17A between subjects with sepsis as opposed to trauma-associated and other forms of ARDS we found subjects with sepsis-associated ARDS had significantly higher levels in both ARDS1 and ARDS2 even after adjustment for severity of illness. (Supplemental Table 5; supplemental digital content 1). We again performed sub-group analyses testing whether the relationship between IL-17A concentration and percentage of alveolar neutrophils differed between trauma and sepsis-associated ARDS. In ARDS1, we found that BAL IL-17A concentration was associated with higher percentage of alveolar neutrophils in both sepsis-associated and trauma associated ARDS (Table 4). In contrast, serum IL-17A concentrations were only associated with percentage of alveolar neutrophils in sepsis-associated ARDS ($p=0.03$). More than half of the patients with sepsis in ARDS1 had pneumonia (N=36).

IL-17A is associated with poor outcome in ARDS

We next sought to identify associations between IL-17A and poor outcomes (organ dysfunction and death) in patients with ARDS. In both ARDS1 and the ARDS2 validation cohort, a two-fold increase in circulating IL-17A was associated with increasing SOFA score in an unadjusted model (ARDS1: $\beta=0.33$; $p=0.04$, ARDS2: $\beta=0.32$; $p=0.03$) and when adjusted for race/treatment group (ARDS1 only), age, gender, and sepsis (ARDS1: $\beta=0.40$; $p=0.03$; ARDS2: $\beta=0.36$; $p=0.02$, Table 5). Notably, inclusion of serum IL-8 levels in the multivariate models extinguished the association observed between IL-17A and SOFA score in both ARDS1 and ARDS2.

Associations between circulating IL-17A levels and 28-day mortality were less consistent than with SOFA score. We did not find an association between circulating IL-17A levels and 28-day mortality in ARDS1. Circulating IL-8 was also not independently associated with mortality in ARDS1. In ARDS2, a two-fold increase plasma IL-17A was associated

with higher 28 day mortality (OR (95%CI): 1.36 (1.07-1.71); p=0.01; Table 5). IL-17A remained associated with 28 day mortality (1.45 (1.12-1.88); p=0.005) after adjustment for differences in age, gender, and ARDS risk factor of sepsis. IL-8 was independently associated with mortality in ARDS2 (p=0.01). Inclusion of serum IL-8 levels in the multivariate models again extinguished the association observed between IL-17A and mortality (OR 1.34 (0.99-1.82), p=0.053).

DISCUSSION

Our knowledge of the true factors that initiate and amplify alveolar inflammation in ARDS remains incomplete. A better understanding of the mechanisms driving this inflammation may lead to novel treatment strategies. Here we provide the first evidence linking IL-17A with ARDS in critically ill patients. We have used a population of critically ill subjects with ARDS from which circulating and BAL fluid samples were obtained simultaneously early in the course of disease to show that increasing circulating and BAL IL-17A levels are associated with increased lung inflammation as represented by percentage of alveolar neutrophils. In sub-group analyses we showed that IL-17A levels were higher in patients with sepsis-associated relative to trauma-associated ARDS, but the relationship between elevated BAL IL-17A and increased percentage of alveolar neutrophils remained strong in both groups. Elevated BAL IL-17A was also significantly associated with an elevated percentage of alveolar neutrophils in both direct or indirect ARDS. Evidence that this association might have an effect on alveolar permeability is shown by the fact that increasing BAL IL-17A is also strongly associated with total alveolar protein, a measure of increased alveolar permeability. Taken together, these findings are consistent with a model in which IL-17A helps to drive neutrophilic inflammation in the lungs of ARDS patients and that this could lead to protein leak and alveolar filling. This model is supported by findings in murine models of lung infection and injury (10, 12, 13, 28), and parallels the association seen between IL-17A levels and neutrophil counts in clinical studies of asthma and cystic fibrosis (18, 37).

Previous work has focused on the role of IL-8 as an important chemoattractant in ARDS (23, 24). However, IL-17A is also thought to be an important mediator of neutrophilic inflammation and host response in murine models of lung infection and injury and IL-17A has been shown to induce expression of IL-8 in respiratory epithelium (38). This suggests that IL-17A could work upstream or in concert with IL-8 to promote alveolar neutrophil recruitment. As would be predicted from prior work, increasing BAL IL-8 levels were also strongly associated with an increased percentage of alveolar neutrophils in our study. However, in spite of this expected relationship between IL-8 and percent neutrophils, the association between BAL IL-17A levels and alveolar percent neutrophils remained significant after adjustment for IL-8 levels. Taken together these findings suggests that IL-17A may be an important mediator of neutrophil recruitment and alveolar injury in ARDS and that these effects are, in part, independent of IL-8. Elucidation of the local mechanisms of action of IL-17A may help identify novel treatment strategies.

Our results demonstrated that alveolar and systemic IL-17A levels are highly associated with alveolar inflammation and importantly, that this relationship has implications for

patient outcomes. In both ARDS1 and ARDS2, elevated IL-17A was associated with higher SOFA scores. In the larger ARDS2 group, we found that higher levels of circulating IL-17A were associated with increased 28 day mortality. This finding was not replicated in ARDS1 but this may have been due to the smaller sample size and the inclusion of trauma-associated ARDS. It may have also been related to the difference in sampling times (within 48 hours of ARDS onset in ARDS1 and 48 hours in ARDS2). Severity of illness based on APACHE II score was also higher in ARDS2. In parallel with our IL-17A findings, IL-8 levels were associated with mortality in ARDS2 but not in ARDS1.

Our work is the first to implicate circulating and alveolar IL-17A with alveolar inflammation in ARDS. However, our findings linking IL-17A levels with outcomes in ARDS are resonant with other recent published findings. In a recently published study, Yu et al. examined the ratio of pro-inflammatory IL-17A producing lymphocytes (Th17 cells) to immunosuppressive regulatory T cells (Treg) found in the peripheral blood of patients with ARDS. They found that a high Th17/Treg ratio was associated with death in ARDS patients (39). In a small group of patients with ARDS due to H1N1 influenza, Hagau et al reported that higher levels of circulating IL-17 were present in patients who died (40). Taken together, our findings extend this prior work implicating IL-17-related pathways in ARDS-related outcomes. Of note, we determined that associations between IL-17A and ARDS outcomes are attenuated by inclusion of IL-8 in the model. This finding suggests that effects of IL-17A and IL-8 on systemic organ dysfunction in ARDS may involve shared mechanisms. The notion that IL-17A may directly induce IL-8 is supported by previous studies (16, 38, 41). Future studies will need to determine to what extent IL-17A might contribute to the pathophysiology of ARDS-related outcomes and how IL-8 might modify or mediate this contribution.

In summary, our studies implicate IL-17A as a potential mediator of alveolar inflammation in ARDS and as a marker of poor outcomes in these patients. However, our study has some important limitations. First, our findings linking IL-17A to alveolar inflammation was done in only a single cohort. Obtaining peripheral blood, alveolar fluid and alveolar inflammatory cells from an independent set of patients with ARDS on a scale done in ARDS1 will be challenging but necessary to confirm these findings. Second, our findings do not identify the source of the IL-17A in the BAL fluid or whether IL-17A might have simply leaked into the alveolar space as a result of neutrophil migration and protein leak. Future studies will seek to identify the individual cells producing IL-17A in the alveolar space of ARDS patients and will sample the alveolar compartment of critically patients at risk for ARDS to see if IL-17A levels in the lung precede the alveolar injury. Third, ARDS in our study subjects was predominantly due to sepsis or pneumonia, and to a much lesser extent, trauma. Thus, we do not yet know if our findings are generalizable to all forms of ARDS. Future work will need to address these issues to better understand this potentially important finding.

CONCLUSIONS

Circulating and alveolar IL-17A concentrations are closely linked with alveolar neutrophilic inflammation in ARDS. This relationship between IL-17A and the degree of alveolar inflammation is independent of IL-8 levels. Elevated circulating IL-17A is associated with

increased organ dysfunction in patients with ARDS. To our knowledge, these are the first studies linking IL-17A with alveolar inflammation and organ dysfunction in patients with ARDS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Subject Characteristics

Characteristic	ARDS1 (n= 86)	ARDS2 (n=140)
Patient Age, mean ± SD	50 ± 17	54 ± 16
Male patients, no. (%)	54 (63)	104 (74)
Caucasian, no. (%)	76 (88)	140 (100)
ARDS risk factor, no. (%)[a]		
Sepsis	56 (65)	113 (81)
Pneumonia	36 (42)	63 (45)
Trauma	34 (39)	0 (0)
Aspiration	17 (20)	33 (23)
Inhalation	1 (1)	0 (0)
Drowning	1 (1)	0 (0)
Other	10 (12)	46 (33)
APACHE score, mean ± SD	22 ± 6	26±9
Comorbidities, no. (%)		
Diabetes	18 (21)	34 (24)
Cirrhosis	6 (7)	16 (11)
Chronic Renal Insufficiency	2 (2)	9 (6)
Circulating IL-17A pg/mL, Median (IQR)	3.5 (1.6-9.6)	3.7 (1.4-11.5)
28-day Mortality, no. (%)	13 (15)	26 (18)
SOFA score, mean ± SD[b]	9 ± 4	7 ± 3

ARDS = Acute Respiratory Distress Syndrome; APACHE = Acute Physiology and Chronic Health Evaluation; SD = standard deviation; IQR = interquartile range; IL-17A = interleukin-17A.

[a] Risk factors for ARDS are not mutually exclusive

[b] Highest SOFA score in first 7 days

Table 2

Association of early IL-17A levels with percentage of alveolar neutrophils

Log_2(IL-17A)	N	β (95% CI) [a]	p	β (95% CI) [b]	p
BAL IL-17A	80	5.7 (3.5-7.9)	<0.001	6.1 (3.6-8.5)	<0.001
Serum IL-17A	83	3.7 (0.9-6.5)	0.01	3.7 (0.5-7.0)	0.02
BAL IL-8	80	7.4 (4.9-10)	<0.001	7.4 (4.8-10)	<0.001
Serum IL-8	83	1.7 (−3.8-7.2)	0.53	2.1 (−4.5-8.8)	0.52

BAL = bronchoalveolar lavage; APACHE = Acute Physiology and Chronic Health Evaluation; IL-8 = Interleukin-8, IL-17A = Interleukin 17-A. β represents increase in percentage of alveolar neutrophils for each two fold increase in IL-17A concentration.

[a] Linear regression between log_2 transformed day 0 IL-17A concentration and percent alveolar neutrophils

[b] Linear regression adjusted for age, gender, race, treatment group, sepsis and APACHE II score

Table 3

Association of variables with percent alveolar neutrophils in multivariate model in ARDS1

Variables	P Value[a]	β Coefficient	95% CI
BAL IL-17A	0.001	4.2	1.9 – 6.6
BAL IL-8	<0.001	5.8	3.1 – 8.4
Age	0.32	0.18	−0.2 - 0.5
Group	0.85	−1.06	−12 - 10
Male	0.55	3.55	−8.4 - 15
Race	0.39	3.01	−4.0 - 10
APACHE II	0.89	0.06	−0.8 - 0.9
Sepsis	0.91	0.75	−12 - 13

β = Beta coefficient for linear regression; CI = confidence interval; BAL = Bronchoalveolar lavage; IL-17A = Interleukin-17A; IL-8 = Interleukin-8; Group = treatment group (Fish oil versus placebo); APACHE = Acute Physiology and Chronic Health Evaluation.

[a]Linear regression between $\log_2$ transformed IL-17A and percent alveolar neutrophils adjusted for $\log_2$ transformed IL-8, age, treatment group, gender, race, APACHE II, and sepsis

Table 4

Association of IL-17A levels and percentage of alveolar neutrophils in subjects with sepsis or trauma-associated ARDS

Log_2(IL-17A)	N	β (95% CI) [a]	p
BAL IL-17A			
Trauma/Other	28	13 (6.8-19)	<0.001
Sepsis	52	4.7 (2.1-7.3)	0.001
Serum IL-17A			
Trauma/Other	29	NS	0.40
Sepsis	54	3.46 (0.43-6.5)	0.03

CI = confidence interval; BAL = Bronchoalveolar lavage; IL-17A = Interleukin-17A; NS = non-significant.

[a] Linear regression between log-2 transformed day 0 IL-17A and percent alveolar neutrophils. β represents increase in percentage of alveolar neutrophils for each two fold increase in IL-17A concentration.

Table 5

Association between IL-17A concentration and Clinical Outcomes in ARDS

Group	SOFA β [a]	p	SOFA Adj β [b]	Adj p [b]	Mortality OR (95% CI) [c]	p [c]	Mortality Adj OR (95%CI) [d]	Adj p [d]
ARDS1								
Serum IL-17A	0.33	0.04	0.40	0.03	--	NS	--	NS
BAL IL-17A	--	NS	--	NS	--	NS	--	NS
ARDS2								
Plasma IL-17A	0.32	0.03	0.36	0.02	1.36 (1.07-1.72)	0.01	1.45 (1.12-1.88)	0.005

β = Beta coefficient for linear regression; OR = odds ratio per two-fold increase in IL-17A concentration; CI = confidence interval; NS = non-significant.

[a] Linear regression between $\log_2$ transformed IL-17A concentration and highest SOFA score in the first 7 days

[b] Linear regression adjusted for ARDS 1: Age, gender, race, treatment group, sepsis or ARDS 2: Age, gender, sepsis

[c] Logistic regression between $\log_2$ transformed IL-17A and 28 day mortality

[d] Logistic regression adjusted for ARDS 1: Age, gender, race, treatment group, sepsis or ARDS 2: Age, gender, sepsis