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Overexpression of CREMα in T Cells Aggravates Lipopolysaccharide-Induced Acute Lung Injury

Eva Verjans,*† Kim Ohl,* Yin Yu,* Ralph Lippe,‡ Angela Schippers,* Anastasia Wiener,* Johannes Roth,‡# Norbert Wagner,* Stefan Uhlig,† Klaus Tenbrock,*# and Christian Martin†#

Transcription factor cAMP response element modulator (CREMα) contributes to various cellular and molecular abnormalities in T cells, including increased IL-17 and decreased IL-2 expression. For development of acute lung injury (ALI), the invasion and regulation of immune cells are highly important, but the role of T cells remains unclear. In this study, we show that CREMα is upregulated in LPS-induced ALI. During the early phase of ALI (day 1), T cell–specific CREMα overexpression enhances the numbers of T cells and expression of TNF-α in bronchoalveolar lavage fluid and deteriorates lung functions. On day 3 of ALI, CREMα transgenic mice present a stronger inflammatory response with higher levels of TNF-α, IL-6, and IL-17 correlating with increased numbers of T cells and neutrophils in bronchoalveolar lavage fluid, whereas expression of Foxp3 and IL-2 and numbers of regulatory T cells are decreased. These changes result in restricted lung function in CREMα transgenic mice. Finally, an adoptive transfer of CREMα−/− CD4+ T cells, but not of wild-type T cells into RAG-1−/− mice results in ameliorated disease levels. Thus, levels of CREMα in T cells determine the outcome of ALI and CREMα transgenic animals represent a model in which proinflammatory T cells aggravate ALI in different phases of the disease. Given the fact that patients with autoimmune diseases like systemic lupus erythematosus show higher levels of CREMα and an increased susceptibility toward infectious complications, our finding is of potential clinical significance and may enable new therapeutic strategies. The Journal of Immunology, 2013, 191: 1316–1323.

Acute lung injury (ALI) is a life-threatening disease characterized by the rapid onset of severe respiratory failure with acute dyspnea, tachypnea, and tachycardia (1, 2). It is mostly associated with sepsis, pneumonia, and polytrauma (3). ALI is characterized by pulmonary edema and the invasion of hematopoietic cells, neutrophils, and monocytes in the initial phase and lymphocytes at later stages. These leukocytes bear TLR-4, whose activation by bacterial LPS drives pulmonary inflammation (4, 5). Hence, a frequently used experimental model for ALI is the instillation of LPS into the airways of animals (6).

Clinically, the acute inflammation may either resolve or progress into a fibroproliferative phase. At the time of these pathophysiologically crossroads, T lymphocytes migrate into the lungs and apparently regulate the resolution of ALI (7, 8). Among the lymphocytes, regulatory T cells (Tregs) appear to promote the resolution of ALI by secretion of TGF-β and further mechanisms (7).

Prior studies demonstrated that RAG-1−/− mice, which have no mature B and T lymphocytes, showed decreased numbers of neutrophils in bronchoalveolar lavage on the second day of ALI compared with mice with a complete immune system, suggesting a disease-modifying effect of T cells already in the first 2 d of ALI (8). However, data that define the role of T cells during the early phase of ALI are not available hitherto (4, 5, 9).

One well-known regulatory mechanism of inflammation involves the second messenger cAMP, which was shown to be induced by LPS in the lung and might contribute to the resolution of inflammation (10). cAMP leads to activation of the protein kinase A (11, 12), which in turn phosphorylates and thus activates the transcription factors CREB and cAMP response element (CRE) modulator (CREMα), which do both bind to CREs in the promoter region of several target genes (13, 14). CREMα is overexpressed in T cells from patients with systemic lupus erythematosus (SLE), resulting in complex biochemical abnormalities within these cells, including enhanced IL-17 and decreased IL-2 expression (15, 16). SLE patients suffer from increased susceptibility to infections that promote ALI, including sepsis (17), which might be related to CREMα-dependent suppression of IL-2 production (15, 18). As yet, the role of CREMα in ALI or inflammatory disorders other than SLE is unknown. We have recently generated a transgenic mouse with a selective overexpression of CREMα in T cells. These T cells are characterized by enhanced expression of IL-17 and IL-21 and decreased expression of IL-2, thus resembling Th17 cells (16). Additionally, these cells share similarities with the phenotype of human lupus T cells.

It was our aim to examine the potential role of these proinflammatory cells in a model of LPS-induced lung injury. We observed a striking aggravation of lung injury in CREMα-overexpressing mice.

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Abbreviations used in this article: Ach, acetylcholine; AHR, airway hyperresponsiveness; ALI, acute lung injury; BALF, bronchoalveolar lavage fluid; CRE, cAMP response element; CREM, CRE modulator; SLE, systemic lupus erythematosus; Treg, regulatory T cell; wt, wild-type.

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already at day 1 of ALI and a worsening until day 3, which manifested with enhanced expression of TNF-α, IL-6, and IL-17 and deteriorated lung functions. To further demonstrate the critical role of CD4+ T cells, we transferred CREM+/− CD4+ T cells into RAG-1−/− mice and showed that genetic deletion of CREM in these cells was protective in ALI. Thus, constitutive and naive proinflammatory T cells represent a risk factor for the severity of LPS-induced ALI.

Materials and Methods

Animals

Experiments were performed with 8- to 12-wk-old wild-type (wt) and CREMα-overexpressing male FVB mice (Harlan) (16, 19) and CREM−/− and RAG-1−/− C57BL/6 mice. CREM−/− mice were a gift of G. Schütz (Heidelberg, Germany). All mice were bred in our animal facility and kept under specific pathogen-free conditions. The wt and transgenic mice were age matched for all experiments. This study was approved by regional governmental authorities, and animal procedures were performed according to the German animal protection law.

Experimental design

Anesthetized mice were intratracheally instilled with a LPS (Escherichia coli O111:B4; Sigma-Aldrich) aerosol (4 mg/kg) via a microsprayer (PennCentury). Control animals received NaCl, and physiological parameters (body weight and temperature) were monitored. After 24 or 72 h, mice were tracheotomized with a 20 G cannula and connected to the ventilator. All mice were mechanically ventilated with a tidal volume of 10 ml/kg and a positive end-expiratory pressure of 2 cm H2O using the flexivent (SCIREQ) ventilation setup. Lung functions were measured by the forced oscillation technique. Airway hyperresponsiveness (AHR) was provoked with nebulized acetylcholine (Ach).

Transfer experiments

Spleen cells from age-matched, donor CREM−/−, and wt mice were pooled and then negatively selected for CD4+ T cells using a commercially available MACS kit (Miltenyi Biotec). Flow cytometry confirmed cell enrichment with 97 ± 1% T cells. Equal numbers of cells from either CREM−/− or wt donors were then injected via the tail vein of RAG-1−/− mice (2 × 106 cells/mouse) 72 h prior to LPS application. On day 3 after injury, cells were collected from lung, lymph node, and spleen of transferred RAG-1−/− mice for flow cytometry. Only mice, in which transferred cells could be detected, were used for analysis.

Wet/Dry ratio and bronchoalveolar lavage

Following ventilation, lungs were removed. The postcaval lobe of the right lung was used to determine the wet/dry ratio to quantify edema formation. The entire right lung was used for bronchoalveolar lavage fluid (BALF) by instilling 700 μl ice-cold PBS.

Lung histopathology

Paraffin-embedded sections (3 μm) from left lung were stained with H&E. Histopathology was evaluated with a scoring system based on local and diffuse cell infiltration, alveolar septal thickening, alveolar congestion, and alveolar hemorrhage.

FACS analysis

A total of 30 μl BALF and 170 μl PBS/0.5% BSA was taken without staining to calculate absolute numbers of BALF cells with the FACScanto (BD Biosciences). The rest of BALF was centrifuged for 10 min at 1250 × g, and the pellet was resolved in 1 ml PBS/0.5% BSA to wash the cells for a second time. Cells were stained with Abs diluted in PBS/0.5% BSA for 20 min at 4°C. For detection of T cells and the T cell subset CD4+, CD4-PE (eBioscience) and CD3-allophycocyanin (eBioscience) were used. Neutrophil granulocytes were stained with Gr-1-FITC (Immuno Tools). A minimum of 10,000 events was collected for evaluation. To identify Tregs, intracellular Foxp3 staining (eBioscience) following manufacturer’s instructions was used and CD4+ T cells were gated to calculate the amount of Foxp3+ cells in this population.

Cytokine measurements

Murine IL-6, TNF-α, IL-17A, and IL-21 were analyzed in supernatants of BALF samples with sandwich ELISAs, according to manufacturer’s protocols (R&D Systems/eBioscience).

RNA isolation and real-time PCR

Murine and human RNA processing and real-time PCR were performed. Relative quantifications of gene expression were calculated with the LightCycler480 software 1.5.0 (Roche). Murine PCR primer sequences were as follows: CREMα forward (5′-AAC TGT CCT CTG ATG CCG TAT G-3′), CREMα reverse (5′-TTG CCC CTT GCT AGT ATG TTA T-3′), IL-2 forward (5′-CCT AGA CAT CGC AGA G-3′), Foxp3 forward (5′-GGC AAA TGG AGT CCG CAA GTG-3′), Foxp3 reverse (5′-CAG GAT AGT ATC TGG TGC GCA-3′), IL-17A forward (5′-AGC TGG ACC ACC ACA TGA ATT C-3′), and IL-17A reverse (5′-CCA CAC CCA CCA GCA TCT TC-3′). Values were normalized to RPS29.

Statistical analysis

All data are presented as mean ± SEM (SEM). Differences between two groups were tested using unpaired two-sided Student’s t test given that data were normally distributed. Comparisons with more than two groups were analyzed with one-way ANOVA and the Tukey posttest. Graph generation and statistical analysis were performed by using GraphPad Prism version 5.0 (GraphPad Software) or JMP 7.0.1 (SAS Institute). *p < 0.05, **p < 0.01, ***p < 0.001.

Results

Intratracheal LPS instillation results in enhanced CREM expression in the lung

It has been suggested that T cells contribute to the development and resolution of experimental ALI by CAMP-dependent mechanisms (10). To investigate whether CREMα, which is a CAMP-dependent molecule, is upregulated during ALI, we instilled LPS intratracheally into wt and CD2-CREMA transgenic mice, which overexpress CREMα in T cells. On day 3, intratracheal LPS instillation enhanced the expression of CREMα in the lungs of wt mice, and even more in CD2-CREMA transgenic mice (Fig. 1), suggesting that most of the CREMα expression relates to the influx of T cells into the lungs. Thus, CREMα expression rises during the acute phase of ALI.

CREMα overexpression in T cells aggravates body weight loss and temperature drop in ALI

We have recently shown that overexpression of CREMα alters T cells toward a preformed proinflammatory phenotype that, once activated, aggravates disease pathology in models of the adaptive immune system like contact dermatitis and murine lupus (16, 20). To evaluate whether the expression of CREMα in T cells affects the clinical course of ALI, which is thought to be primarily dependent on activation of the innate immune system, we intratracheally instilled LPS into wt and CD2-CREMA mice. After 24 h, wt and CD2-CREMA mice showed a comparable weight loss, but on days 2 and 3 weight loss was more severe in the CD2-CREMA transgenic mice (Fig. 2A). Fever, which in mice manifests as a temper-
In our study, LPS treatment only slightly altered edema formation in wt and CD2-CREMα transgenic animals compared with control mice on day 1 (Fig. 4A). Additionally, CREMα overexpression did not alter wet/dry ratio compared with LPS-treated wt animals.

Infiltrating cells were nearly absent in the BALF of control animals (Fig. 4B–D). LPS treatment caused cellular patterns of early-phase ALI. Twenty-four hours after LPS application, mice were ventilated with the flexiVent setup. Total lung resistance $R_{tot}$ (A), tissue resistance $G$ (B), total lung elastance $E$ (C), and tissue elastance $H$ (D). Results represent means ± SEM (WT $n = 7$ and TG $n = 5$ in both groups). *$p < 0.05$, **$p < 0.001$.

Histopathological analysis revealed a more severe lung injury after LPS instillation in CD2-CREMα transgenic mice than in wt animals (Fig. 6H, 6I).
These data indicate that the elevated CREMα levels in T cells alone are sufficient to aggravate the inflammatory response and SLE during late phase of ALI.

**Aggravated inflammation in late-phase ALI correlates with decreased expression of Tregs in CD2-CREMα transgenic animals**

Recruitment of Foxp3+ Tregs is involved in the resolution of ALI, but not in the acute phase (23). Normally, Tregs reside in the tissue and do not invade the alveoli. Therefore, we measured Foxp3 mRNA levels on day 3 of ALI in the tissue of blood-free perfused lungs. The increased Foxp3 gene expression 72 h after LPS instillation was nearly halved in CREMα transgenic mice compared with wt animals (Fig. 7A). To distinguish between Foxp3+ cells in alveoli versus those in the lung interstitium, we determined intracellular Foxp3 levels in the fraction of CD4+ cells in BALF. Transgenic animals with CREMα-dependent T cell dysregulation showed lower percentages of CD4+Foxp3+ cells (Fig. 7B). However, the strong reduction of Foxp mRNA levels in whole lung tissue cannot be completely explained by a difference of ~10% in the expression of Tregs in the BALF of wt and CD2-CREMα transgenic mice. Therefore, we looked for additional mechanisms and found that T cell–specific overexpression of CREMα reduced the amount of IL-2, which constitutes an indispensible cytokine for Treg viability and function, at both pulmonary mRNA and alveolar protein levels (Fig. 7C, 7D). Vice versa, IL-17A mRNA levels were clearly upregulated in the lung tissue of CD2-CREMα transgenic mice (Fig. 7E). To exclude that Treg expression might already be altered in early phases of ALI, we determined Foxp3 and IL-2 expression in lung tissue of mice killed on day 1 of ALI; however, we could not detect measurable levels of these cytokines and Foxp3 (data not shown).

Therefore, CREMα overexpression in T lymphocytes clearly influences the recruitment of Tregs on day 3 of acute lung injury, but not on day 1.

**Genetic deletion of CREM results in amelioration of ALI**

To evaluate whether a genetic deletion of lymphatic CREM would be protective in ALI, we transferred MACS isolated CD4+ T cells of CREM−/− mice or appropriate wt mice into lymphopenic RAG-1−/− mice. Seventy-two hours after transfer, mice were treated with LPS intratracheally. Again, we analyzed these animals on day 3 after LPS application.

Table I. Basal lung function of WT and TG animals on day 3

<table>
<thead>
<tr>
<th></th>
<th>CD2-CREMα Mice</th>
<th>WT Control</th>
<th>TG Control</th>
<th>WT ALI</th>
<th>TG ALI</th>
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<tbody>
<tr>
<td>Total resistance</td>
<td>0.45 ± 0.01</td>
<td>0.49 ± 0.01</td>
<td>0.55 ± 0.04</td>
<td>0.59 ± 0.02</td>
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<td>Elastance (E)</td>
<td>19.55 ± 0.55</td>
<td>19.88 ± 0.60</td>
<td>24.10 ± 0.09</td>
<td>23.75 ± 1.43</td>
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<tr>
<td>Newtonian resistance (Rn)</td>
<td>0.21 ± 0.01</td>
<td>0.32 ± 0.02</td>
<td>0.24 ± 0.01</td>
<td>0.24 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Tissue resistance</td>
<td>3.02 ± 0.07</td>
<td>3.53 ± 0.19</td>
<td>3.75 ± 0.45</td>
<td>4.00 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>Tissue elastance</td>
<td>17.47 ± 0.91</td>
<td>21.32 ± 0.28</td>
<td>21.48 ± 1.90</td>
<td>19.53 ± 2.90</td>
<td></td>
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</table>

Mice were ventilated with the flexiVent. Total lung resistance (Rtot), total lung elastance (E), Newtonian (central airway) resistance (Rn), tissue resistance (G), and tissue elastance (H) are presented in cmH2Ols/cm. Results represent means ± SEM [for wild-type (WT), n = 5 in both groups and for CD2-CREMα (TG), n = 5].
Genetic deletion of CREM in CD4+ T lymphocytes resulted in decreased weight loss upon LPS instillation (Fig. 8A). On day 3, lung functions had deteriorated in wt more severely than in CREM<sup>−/−</sup>T cell–transferred animals (Fig. 8B–E), as follows: total resistance (R<sub>tot</sub>), peripheral airway resistance (R<sub>P</sub>), tissue resistance (G), total lung elastance (E) were significantly higher in wt T cell–transferred animals. For tissue elastance (H) (Fig. 8E) and resistance of the central airways (R<sub>C</sub>) (data not shown), we observed a visible, but not significant decrease in CREM<sup>−/−</sup>–transferred animals. These data indicate stronger disease activity in wt compared with CREM<sup>−/−</sup> T cell–transferred animals. Moreover, the mice with wt T cells showed increased numbers of BALF cells, in particular neutrophils (Fig. 8F, 8G). Additionally, we found elevated levels of TNF-α and IL-6 in wt compared with CREM<sup>−/−</sup> T cell–transferred mice (Fig. 8H, 8I), whereas IL-17A levels were quite low and not significantly different between the two groups (data not shown).

Together with the data from the CREM<sub>a</sub> transgenic mice, these findings indicate that CREM<sub>a</sub> levels in T cells determine the inflammatory response and influence lung functions during the late phase of ALI.

**Discussion**

The present study unravels a critical role of proinflammatory T cells in the development and the resolution of acute LPS-induced lung injury. We show the following: 1) CREM<sub>a</sub> is upregulated in the lungs during ALI; 2) overexpression of CREM<sub>a</sub> enhances T lymphocyte numbers, TNF-α expression, and bronchoconstriction on day 1; and 3) it aggravates the inflammatory reaction on day 3, including an enhanced influx of proinflammatory neutrophils and T cells; enhanced expression of TNF-α, IL-6, and IL-17A; and decreased expression of Foxp3. The finding that the transfer of CREM-deficient CD4<sup>+</sup> T cells ameliorates disease activity of ALI in RAG-1<sup>−/−</sup> mice demonstrates that the presence of CREM<sub>a</sub> regulates the ability of lymphocytes to execute the resolution of pulmonary inflammation. Our data additionally show that lymphocytes can aggravate the early (day 1) and the late phase (day 3) of ALI, suggesting a crosstalk of the innate and the adaptive immune system during LPS-triggered inflammation of the lung.

ALI is classically characterized as an innate immune response followed by an activation of the adaptive immune system. LPS...
administration induces the activation of neutrophils via TLR-4 signaling and thus leads to secretion of typical early phase cytokines such as TNF-α and IL-6 (4). In the early phase of ALI, influx of T cells is usually low, but it constantly rises and peaks at day 3. Previously, T lymphocytes were considered largely irrelevant for ALI, because nude and RAG-1−/−/ mice showed the same ALI phenotype as wt animals (24, 25). More recently, a critical role of T, but not B, lymphocytes for the resolution of ALI became apparent (26, 27). Our data extend these findings as we show that not only the lack, but also the proinflammatory properties of lymphocytes (CREMα dependent) can exacerbate ALI in the acute as well as during the resolution phase. In addition, CREM levels determine the outcome of ALI, because CREM−/− T cells ameliorate the disease course of ALI. Our transfer experiments with CREM−/− CD4+ T cells in RAG-1−/− mice clearly demonstrate that an exclusive alteration of T cell function modifies disease activity. To our knowledge, this study is one of the first to demonstrate that isolated changes in T cell function can alter cell and cytokine profiles in BALF as well as lung function parameters in ALI. Therefore, our experiments suggest that T cells seem to directly contribute to acute inflammatory processes of the lung. However, one has to keep in mind that, in addition to CD4+ T cells in non-RAG mice, other cells could clearly contribute to ALI, including NK and γδ T cells (28).

**FIGURE 7.** Tregs in ALI on day 3. (A) Foxp3 gene expression in lung tissue of NaCl-instilled control animals and LPS-treated wild-type (WT) and CD2-CREMα mice (normalized to RPS29). (B) FACS analysis of CD4+/Foxp3+ cells in BALF of control and LPS-treated WT and TG (WT n = 4 and TG n = 5 in both groups). (C) IL-2 expression in lung tissue and (D) IL-2 protein in BALF (normalized to RPS29). (E) IL-17A mRNA levels in lung tissue (normalized to RPS29). (A, C, and E) Gene expression is expressed as fold induction relative to controls. (A–E) Results represent means ± SEM (WT n = 6 and TG n = 5 in both groups). *p < 0.05, **p < 0.01, ***p < 0.001.

**FIGURE 8.** Transfer of CREM−/− CD4+ cells in RAG-1−/− mice and ALI on day 3. Body weight loss (A) in RAG-1−/− mice with transferred wild-type (WT) (gray) and CREM−/− (black) CD4+ T cells between days 0 and 3 after intratracheal instillation of LPS. Lung function on day 3: total lung resistance Rtot (B), total lung elastance E (C), tissue resistance G (D), and tissue elastance H (E). Total cell number (F) and neutrophils (G) in BALF of LPS-treated animals counted by FACS analysis. Cytokine levels of IL-6 (H) and TNF-α (I) in BALF supernatants. Results represent means ± SEM (RAG-1−/− with WT cells n = 5 and RAG-1−/− with CREM−/− cells n = 5). *p < 0.05, **p < 0.01.
In our study, invasion of neutrophils into the BALF was reduced in CD2-CREMα transgenic animals on day 1, but after 72 h they were clearly upregulated compared with wt mice, whereas a genetic deletion of CREM resulted in the opposite phenotype. Neutrophil influx during ALI occurs at two phases (29), as follows: one immediate IL-17–independent phase during the first 24 h, and one second IL-17–dependent phase after 3 d. Primarily, T cells are the source of IL-17 during the later phase (29), whereas NK cells and innate lymphoid cells are most probably the source of IL-17 during the first 24 h, which was shown with a model of Klebsiella pneumoniae–induced ALI in IL-17 reporter mice (28).

Moreover, in Rag2−/− mice, the dominant source of IL-17 were innate lymphoid cells in a LPS-induced sepsis model (30). In line with this, reduced numbers of neutrophils in the CD2-CREMα transgenic mice correlate with lower expression of IL-6 and IL-17 in the BALF at that time point, whereas neutrophilia during the late phase might be triggered by enhanced T cell–dependent IL-17 expression in the CD2-CREMα transgenic mice. Of note, numbers of T lymphocytes were enhanced in the BALF of LPS-treated CD2-CREMα transgenic animals at any analyzed time point and clearly correlated with TNF-α expression, which was higher in the CD2-CREMα transgenic mice and on the other side lower in the mice transferred with CREM−/− T cells. LPS-induced TNF-α expression by itself is important for bronchoconstriction and not for recruitment of inflammatory cells per se (22). This might explain why lung function parameters are worsened already at day 1 in CD2-CREMα transgenic mice, whereas wet/dry ratio in these lungs as a marker of capillary leak and inflammatory infiltration did not differ at that time point.

As described previously, patients with SLE, a chronic inflammatory multisystem disorder, show abnormal biochemical T cell functions, which might be related to autoimmunity and to enhanced susceptibility toward infections (31–33). T lymphocytes of these patients show an overexpression of the transcription factor CREMα. CREMα was linked to decreased IL-2 production, possibly due to increased levels of Ca2+/calmodulin-dependent kinase IV, which induces the transcription of CREM. Once activated, CREM binds to promoters of genes like IL-17A and F, the TCRγ-chain, c-Fos, or IL-2 and suppresses or activates their activity (34). In the CD2-CREMα transgenic mice, we were able to mimic many details of the phenotype in human SLE T cells and in particular reduced expression of IL-2 and enhanced expression of IL-17 after T cell stimulation (16). In line with these findings, we found enhanced IL-17A levels in the BALF of LPS-treated CREMα transgenic animals on day 3. In addition, CREMα-overexpressing animals showed higher levels of typical proinflammatory and T cell cytokines on days 1 and 3. Many of these cytokines, such as IL-1β, IL-6, IFN-γ, and TNF-α, have been implicated in the pathogenesis of ALI (6), and most of these cytokines have CRE sequences in their promoter regions. Therefore, it is possible that CREMα contributes to the transcriptional activation of these cytokines in the course of ALI (35, 36), and during late and early stages different promoters are activated.

CREMα overexpression clearly enhanced IL-17A levels in the BALF 72 h after LPS administration. Although IL-17A is also produced by NK cells, mast cells, and neutrophils, the main source of this cytokine in inflamed lungs are T cells, especially Th17 and γδT cells (37). In ALL, IL-17A is primarily upregulated around day 4 (8), and its role is to induce the expression of proinflammatory mediators that are critical for the recruitment of neutrophils to the sites of inflammation (37–39). At day 3 we found that upregulated IL-17A coincided with enhanced cytokine levels and intensified neutrophil recruitment in CD2-CREMα transgenic lungs after LPS challenge.

CREMα overexpression may aggravate ALI by proinflammatory responses, but also through repressed control mechanisms of inflammation, especially at the beginning of resolution. Particularly CD4+Foxp3+ cells (Tregs) as key players for suppression of Ag-driven and T cell–dependent immune responses (40, 41) were affected by CREMα overexpression 72 h after LPS administration. The transcription factor Foxp3 is the best-established marker for Tregs, particularly in mice, and apparently is essential for their immune-suppressive properties (42). In line with this, CD2-CREMα transgenic mice showed reduced Foxp3 expression in their lungs on day 3. In addition, IL-2 was diminished at mRNA and protein level. External IL-2 is necessary for function and to survival of Tregs, because they are not able to produce IL-2 by themselves. As previously described (15), CREMα binds directly to the IL-2 promoter and suppresses its activity, resulting in lower IL-2 expression. We suggest that CREMα suppresses Foxp3 expression and Treg numbers in the lungs via downregulation of IL-2. Alternatively, a direct interaction between a CRE region of the Foxp3 promoter and CREMα is conceivable; however, CD2-CREMα transgenic mice do not show reduced numbers of Tregs per se (16). Tregs markedly downregulate Th17-directed responses and reduce IL-17A levels in pulmonary inflammation (39). Therefore, it seems likely that the upregulation of IL-17A in the lungs of CD2-CREMα transgenic mice results both from lower expression levels of Tregs and additional direct binding of CREMα to the IL-17A promoter.

The present findings suggest that the transcription factor CREMα in T cells can promote acute lung injury. CREMα overexpression causes an aggravated inflammatory phenotype that is related to higher levels of IL-17A and IL-21 and reduced resolution of inflammation via decreased expression of IL-2 and lower levels of Tregs. Vice versa, transfer of CREM−/− CD4+ T cells in Rag1−/− mice clearly reduced disease activity. These results demonstrate that T cells play a major role in lung injury and that proinflammatory T cells, which are abundantly expressed in patients with autoimmune diseases, including SLE patients, are a risk factor for the susceptibility toward ALI. Given the fact that patients with autoimmune diseases have a higher susceptibility toward infectious complications, our finding is of potential clinical interest.

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Disclosures

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