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IL-17, Produced by Lymphocytes and Neutrophils, Is Necessary for Lipopolysaccharide-Induced Airway Neutrophilia: IL-15 as a Possible Trigger

Stephane Ferretti, Olivier Bonneau, Gerald R. Dubois, Carol E. Jones, and Alexandre Trifilieff¹

IL-17 is a cytokine implicated in the regulation of inflammation. We investigated the role of this cytokine in neutrophil recruitment using a model of LPS-induced lung inflammation in mice. In the bronchoalveolar lavage, LPS induced a first influx of neutrophils peaking at day 1, followed by a second wave, peaking at day 2. IL-17 levels were increased during the late phase neutrophilia (day 2), and this was concomitant with an increased number of T cells and macrophages, together with an increase of KC and macrophage-inflammatory protein-2 levels in the lung tissue. Intranasal treatment with a neutralizing murine anti-IL-17 Ab inhibited the late phase neutrophilia. In the bronchoalveolar lavage cells, IL-17 mRNA was detected at days 1, 2, and 3 post-challenge, with a strong expression at day 2. This expression was associated with CD4⁺ and CD8⁺ cells, but also with neutrophils. When challenged with LPS, despite the absence of T cells, SCID mice also developed a neutrophilic response associated with IL-17 production. In BALB/c mice, IL-15 mRNA, associated mainly with neutrophils, was evidenced 1 day after LPS challenge. In vitro, IL-15 was able to induce IL-17 release from purified spleen CD4⁺ cells, but not spleen CD8⁺ or airway neutrophils. We have shown that IL-17, produced mainly by CD4⁺ cells, but also by neutrophils, plays a role in the mobilization of lung neutrophils following bacterial challenge. In addition, our results suggest that IL-15 could represent a physiological trigger that leads to IL-17 production following bacterial infection. *The Journal of Immunology*, 2003, 170: 2106–2112.

Murine IL-17, previously termed CTLA8, is a recently cloned cytokine (1) secreted by activated CD4⁺ cells (2). Its human analog has been cloned (3), and its production has been reported to be associated with activated CD4⁺ cells of the Th0/Th1 phenotype (2, 4). Although IL-17 production seems to be restricted to CD4⁺ cells, its receptor is widely distributed (5) and its activation exerts proinflammatory effects. In vitro, IL-17 stimulated the production of IL-1 β and TNF- α from macrophages (6), induced the production of neutrophil chemoattractants from human bronchial epithelial cells (7, 8), and also regulated the ICAM-1 expression and chemokine production from keratinocytes (9). In vivo, IL-17 has been mainly implicated in granulopoiesis (10, 11) and in the selective recruitment of neutrophils in different tissues (12, 13). To date, IL-17 has been studied principally in rheumatoid arthritis (14, 15), allograft rejection (16, 17), and host defense against lung infection (18, 19).

Chronic obstructive pulmonary disease is characterized by airway obstruction that is slowly progressive and irreversible. This disease has an inflammatory component that is characterized by infiltration of neutrophils and macrophages into the lung (20), and a role for IL-17 in this disease has been recently suggested (21). As such, direct lung instillation of IL-17 in rat (12) or overexpression of IL-17 through adenovirus gene transfer into the lung of mice (19) has been shown to result in a selective recruitment of neutrophils. This neutrophil influx is dependent on the production of proinflammatory cytokines such as IL-1 β , TNF- α , and G-CSF

(19), and also on the induction of CXC chemokines (12). This has been confirmed using IL-17R-deficient mice. Following airway bacterial infection, these mice displayed a delay in neutrophil recruitment into the alveolar space, and this was associated with a significant reduction in levels of G-CSF and macrophage-inflammatory protein-2 (MIP-2)² (18). More recently, increased levels of IL-17 have been reported in healthy volunteers following organic dust exposure (22).

In this study, we investigated the relative importance of IL-17 in the airway inflammation using murine models of LPS-induced lung neutrophilic inflammation, and show a potential role for IL-15 in the induction of IL-17.

Materials and Methods

Animals

Five-week-old female BALB/c and T and B cell-deficient BALB/c C.B.-17 scid/scid mice (SCID) were obtained from Charles River (Margate, U.K.). SCID mice were housed in specific pathogen-free animal facilities. All animals were housed in plastic cages in air-conditioned room at 24°C in a 12-h light-dark cycle. Food and water were available ad libitum. The studies reported in this work conformed to the U.K. Animals (Scientific Procedures) Act 1986. The lack of B and T cells in SCID mice was confirmed by the absence of total serum IgG and the lack of detectable staining (FACS analysis) for CD4 and CD8 Abs in the blood.

LPS-induced lung inflammation

Mice were challenged intranasally with 0.3 mg/kg of LPS (*Salmonella typhosa*; Sigma-Aldrich, Poole, U.K.) in 50 μ l of sterile PBS or with sterile PBS alone, and sacrificed at different time point thereafter, as described previously (23). For the neutralization experiment, BALB/c mice were treated, intranasally, 1 day after the LPS challenge with 200 μ g of a neutralizing anti-murine IL-17 Ab (R&D Systems, Abingdon, U.K.) and sacrificed 24 h later. Control mice received either 200 μ g of the rat IgG2a isotype control or 50 μ l of sterile PBS. In a separate experiment, BALB/c

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² Abbreviation used in this paper: MIP, macrophage-inflammatory protein.

mice were challenged intranasally with 1 μ g of murine IL-17 (R&D Systems) in 50 μ l of sterile PBS or with sterile PBS alone, and sacrificed 6 or 24 h after the challenge.

Assessment of airway inflammatory cells and mediator levels

Bronchoalveolar lavage differential cell counts and mediator measurements were performed, as described previously (23–25). TNF- α , IL-17, IL-1 β , KC, and MIP-2 levels were measured using commercially available ELISA kits (R&D Systems). The limit of sensitivity of these assays was 2–5 pg/ml.

Cell sorting

BALB/c mice were challenged with 50 μ l of PBS ($n = 10$) or 0.3 mg/kg of LPS ($n = 20$) and sacrificed 1, 2, or 3 days after. Bronchoalveolar lavage from PBS-challenged animals was pooled and RT-PCR analysis for IL-17 mRNA was performed, as described below. Bronchoalveolar lavage from LPS-challenged animals, obtained 2 and 3 days after the challenge, was pooled and washed twice ($400 \times g$ for 10 min) with the staining buffer (PBS containing 10% mouse serum and 2 mM EDTA) before immunostaining for CD4 and CD8 using the following Abs purchased from BD Pharmingen (San Diego, CA): anti-CD4 (FITC conjugated, clone RM4-5); anti-CD8 (PE conjugated, clone 53-6.7). Nonspecific binding was blocked by incubating cells at 4°C for 30 min in the staining buffer. Cells were then incubated for an additional 30 min with Abs (1 μ g/ 10^6 cells) and washed twice. Total cells were then sorted into CD4⁺ (99% pure), CD8⁺ (99% pure), and CD4/CD8 double negative. The double-negative population was then incubated onto petri dishes, at 37°C in a CO₂ incubator (5% CO₂, 95% air), using RPMI supplemented with 10% FCS as a medium. After 2 h, neutrophils in the supernatant (98.5% pure) were isolated from adherent macrophages (99% pure). Due to the low numbers of lymphocytes present in the bronchoalveolar lavage cells 1 day after the LPS challenge, total lymphocytes (CD3⁺ cells) were sorted, and the remaining population was purified into neutrophils and macrophages, as described above.

RNA isolation, cDNA synthesis, and RT-PCR

Total RNA was isolated from bronchoalveolar lavage-derived cells using the RNeasy mini RNA isolation kit (Qiagen, Crawley, U.K.), according to the manufacturer's instructions. For RT-PCR analysis, first strand cDNA was synthesized from 0.25 μ g of total RNA in a total reaction volume of 20 μ l using random primers, reagents, and conditions supplied in the first strand cDNA synthesis kit for RT-PCR (avian myeloblastosis virus) from Roche Molecular Biochemicals (Lewes, U.K.). For PCR, each reaction mixture contained 0.2 mM dNTPs and 1 \times PCR buffer containing 1.5 mM MgCl₂, 0.25 U Taq DNA polymerase (Roche Molecular Biochemicals), 50 pmol each primer, 4 μ l of first strand cDNA, and deionized water in a 25 μ l reaction volume. Murine β -actin RT-PCR primers were purchased from Stratagene (Amsterdam Zuidoost, The Netherlands) and gave a PCR product of the expected size, 514 bp. Other primers were designed from published sequences, as follows: IL-17 primer, forward, 5'-GGT CAA CCT CAA AGT CTT TAA CTC-3'; reverse, 5'-TTA AAA ATG CAA GTA AGT TTG CTG-3'. The expected size of the IL-17 PCR product was 399 bp. IL-15 primers were forward, 5'-GTG ACT TTC ATC CCA GTT GC-3'; reverse, 5'-TCA CAT TCC TTG CAG CCA GA-3'. The expected size of the IL-15 PCR product was 180 bp. Cycling conditions for amplification for IL-17 were as follows: 94°C for 105 s, annealing for 15 s, 72°C for 30 s for 1 cycle, followed by 34 or 39 cycles of 94°C for 15 s, annealing for 15 s, and 72°C for 30 s. Annealing for all the primer pairs was conducted at 50°C, except β -actin, which used an annealing temperature of 60°C. Amplification of IL-15 was performed over 32 cycles and β -actin over 26 cycles. PCR products were analyzed on 1.5% agarose gels and stained with ethidium bromide.

Neutrophil and lymphocyte isolation and stimulation

Neutrophils and CD4⁺ and CD8⁺ cells were isolated by high-gradient magnetic cell separation (MACS; Miltenyi Biotec, Bergisch-Gladbach, Germany), according to the manufacturer's instructions. Neutrophils were isolated from bronchoalveolar lavage obtained from 10 BALB/c mice 1 day after LPS challenge. All the lavages were pooled, and the cells were washed twice in PBS containing 1% BSA. B and T cells were depleted using CD90 and CD45R mAbs coupled to magnetic beads and neutrophils purified from macrophages by adherence, as described above. Spleens, obtained from five naive BALB/c mice, were used to purify CD4⁺ and CD8⁺ cells. Mononuclear cells were isolated from suspended splenocytes by density-gradient centrifugation using Isopaque-Ficoll separation medium and resuspended in PBS containing 1% BSA. CD4⁺ and CD8⁺ cells were isolated using CD4 and CD8 mAbs coupled to magnetic beads.

The purified cells were washed twice in PBS before being adjusted to the required concentration (2×10^5 cells in 300 μ l) in RPMI 1640 containing 10% mouse serum. Neutrophils and T cells were cultured in 48-well plates at 37°C under a 5% CO₂, 95% air atmosphere, in the presence or absence of LPS (*S. typhosa*; Sigma-Aldrich) or IL-15 (R&D Systems) for 24 or 48 h, in triplicate. Cell supernatants were collected, centrifuged ($2000 \times g$ for 5 min), and stored at -80°C before being analyzed for cytokine levels, as described above.

Data analysis

Results are expressed as individual data and means or mean \pm SEM. Statistical significance ($p < 0.05$) was determined using a two-tailed Student's *t* test for unpaired data or a Mann-Whitney test with Bonferroni correction for multiple comparison for in vitro and in vivo experiments, respectively.

Results

Time course of LPS-induced lung inflammation

In the BALB/c mice, LPS-induced bronchoalveolar lavage inflammatory cell infiltration was followed up to 15 days after the challenge (Fig. 1). An early neutrophil influx was observed, peaking at day 1 before decreasing and plateauing at 30 and 36 h. This early neutrophil influx was followed by a second wave peaking at day 2 and going back to baseline by day 10. Macrophage numbers were below control levels at 3 and 6 h before coming back to baseline at day 1 and increased by day 2. Macrophage numbers were maximal at day 4 and still above control levels at day 15. A biphasic influx of lymphocytes was observed, with a first peak at day 1 and a second large peak at day 4. This second wave was still above control levels at day 15. At all the time points, no significant eosinophil infiltration was observed following the LPS challenge (data not shown).

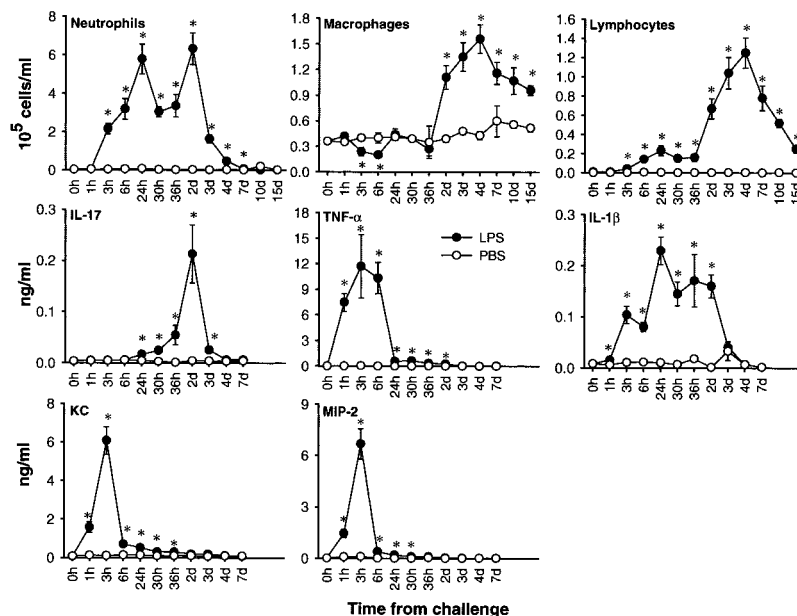
The time course of LPS-induced production of bronchoalveolar lavage cytokines and chemokines was also studied (Fig. 1). IL-17 production started at day 1, peaked at day 2, and came back to baseline by day 4. TNF- α levels were increased at the first time point examined, 1 h, and were sustained up to 6 h, after when levels were dramatically reduced, but were still significantly elevated up to day 2. IL-1 β was present from 1 h postchallenge and peaked at day 1, and the production was sustained up to day 2. MIP-2 and KC were produced as early as 1 h, peaked at 3 h, and came back to baseline by 30 and 48 h, respectively. Because all the chemokines/cytokines were back to baseline by days 4 and 7, the late time points (days 10 and 15) were not studied. At all the time points, PBS challenge had no significant effect on any of the parameters measured when compared with naive animals (time, 0 h) (Fig. 1).

IL-17 neutralization experiment

Because the peak production of bronchoalveolar lavage IL-17 coincided with the second wave of neutrophils, we asked the question as to whether IL-17 could be involved in the late neutrophil influx. To ensure that the situation seen in the bronchoalveolar lavage was a reflection of the whole lung, the kinetic profile for IL-17, KC, and MIP-2 was studied in the lung tissue. A small, but significant increase of IL-17 was only observed 48 h after the challenge (Fig. 2). In agreement with the bronchoalveolar lavage data, lung MIP-2 and KC levels peaked at 3 h before decline by 24 h. However, the level of both chemokines further increased 48 h after the challenge (Fig. 2).

Having established that IL-17 was not produced before 24 h postchallenge, BALB/c mice were intranasally treated with a neutralizing anti-IL-17 Ab 1 day after the LPS challenge, and bronchoalveolar lavage was performed 2 days after the LPS challenge. When compared with PBS-treated animals, the anti-IL-17 Ab reduced the neutrophil influx by 50%, but did not significantly affect

FIGURE 1. Time course of bronchoalveolar lavage inflammatory cell infiltration and cytokine and chemokine production following LPS challenge. Mice were intranasally challenged with LPS or PBS and sacrificed at different time points for bronchoalveolar lavage. Data are expressed as mean \pm SEM of two to four different experiments, and each included six to eight mice per group. Significance, indicated by *, is vs PBS-challenged animals at the same time point.



the other cell type (Fig. 3A). In agreement with the published literature, suggesting that IL-17 attracts neutrophils via the production of KC and MIP-2 (12, 13), the neutralizing anti-IL-17 Ab treatment inhibited the lung levels of both chemokines (Fig. 3B). The isotype control, rat IgG2a, had no significant effect on any of the parameters measured.

IL-17-induced airway neutrophil influx

To further demonstrate that IL-17 is able to attract neutrophils in murine airways, the effect of direct instillation of 1 μ g of IL-17 into BALB/c mice lungs was studied. When compared with PBS, IL-17 induced a significant neutrophil influx 24, but not 6, h after the instillation. In agreement with the data obtained in the LPS model, no production of KC and MIP-2 was observed in the bronchoalveolar lavage at both time points (data not shown). However, a significant production of both chemokines could be evidenced in the lung 24 h after IL-17 instillation (Fig. 4).

Determination of IL-17-producing cells

The bronchoalveolar lavage cell type responsible for the IL-17 production in BALB/c mice was studied at the mRNA level by RT-PCR. In a first experiment, IL-17 mRNA expression was studied 2 days after the challenge (Fig. 5). Following LPS challenge, a strong signal was observed in the total cell population, and in the CD4⁺ and CD8⁺ cells. Unexpectedly, IL-17 mRNA was also associated with the CD4/CD8 double-negative cells (i.e., macrophages and neutrophils). The latter population was then separated into neutrophil and macrophages and subjected to RT-PCR analysis for IL-17. As shown in Fig. 5, IL-17 mRNA was associated with neutrophils, but not macrophages, at day 2. Due to the low number of lymphocytes recovered at day 1, it was not possible to

sort this population into CD4- and CD8-positive cells, and therefore the whole lymphocyte population was studied (CD3⁺ cells). When compared with day 2, IL-17 mRNA was associated only with the CD3⁺ cells (Fig. 5). At day 3 postchallenge, although a faint band was observed in the total LPS cell population, no IL-17 mRNA could be detected in any of the sorted cell types, probably reflecting a very low level of expression at this late time point (Fig. 5).

SCID mice produce IL-17

To further demonstrate that, in vivo, cells other than lymphocytes could be a source of IL-17 at the protein level, SCID mice were challenged with LPS and bronchoalveolar lavage inflammation was studied at 24, 30, and 48 h postchallenge. When compared with BALB/c mice, similar numbers of neutrophils were present at 24 h (Fig. 6A). In contrast, the late phase neutrophilia was abolished and, when compared with day 1, a 2-fold decrease in the neutrophil number was observed at day 2 ($p = 0.025$) (Fig. 6A). No lymphocytes were found in the bronchoalveolar lavage, and the LPS challenge had no influence on the macrophage numbers. Despite the absence of lymphocytes, a significant increase in IL-17 production in the bronchoalveolar lavage was observed at all the time points studied (Fig. 6A). Lung IL-17 levels were significantly increased only at 48 h (Fig. 6B). KC and MIP-2 levels could not be detected in the bronchoalveolar lavage fluids (not shown), but were found in the lungs at all the time points (Fig. 6B).

IL-15 mRNA is produced following LPS challenge and induced IL-17 production in vitro

IL-15 has been shown to trigger IL-17 secretion from human PBMC in vitro (26); therefore, we asked the question as to whether

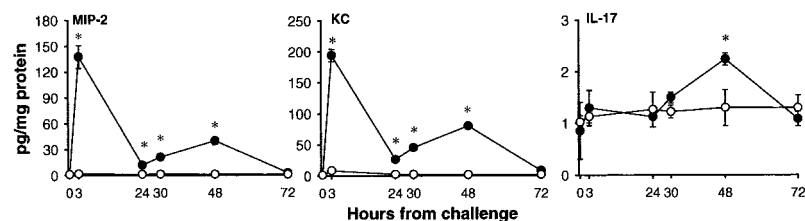
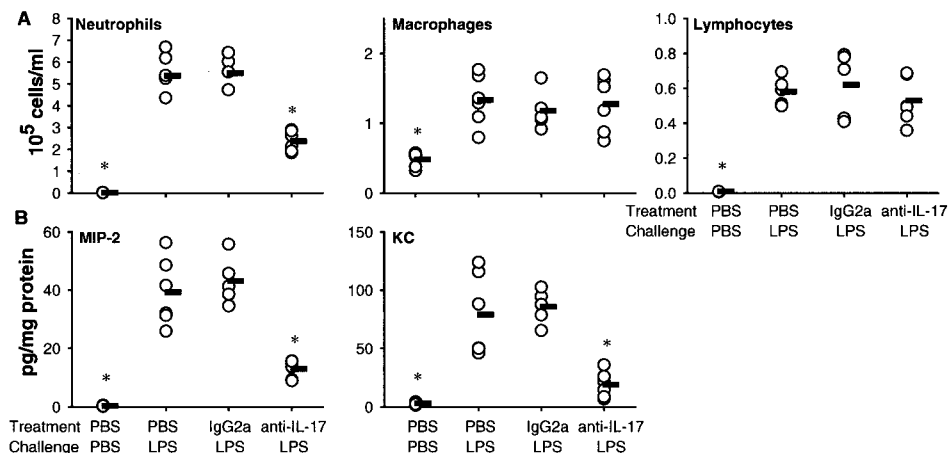


FIGURE 2. Time course of lung IL-17 and chemokine levels after LPS challenge. Mice were intranasally challenged with LPS or PBS, and lungs were processed for chemokine measurement at different time points. Data are expressed as mean \pm SEM of two to three different experiments, and each included 10 mice per group. Significance, indicated by *, is vs PBS-challenged animals at the same time point.

FIGURE 3. Effect of a neutralizing anti-IL-17 Ab on the late phase inflammation. Mice were intranasally challenged with LPS or PBS. Twenty-four hours after, mice were treated with 200 μ l of a neutralizing anti-IL-17 Ab, or the isotype control, or 50 μ l of PBS. Bronchoalveolar lavage was performed 2 days after the challenge, and lungs were processed for chemokine measurement. Individual data, from five to six mice per group, and the mean (horizontal bar) are shown. Significance, indicated by *, is vs PBS-treated/LPS-challenged animals.



in our model the production of IL-17 could be associated with this cytokine. Indeed, in BALB/c mice 1 day after the LPS challenge, a strong expression of the IL-15 mRNA was associated with the neutrophil population (Fig. 7A).

We next investigated, *in vitro*, whether IL-17 protein could be induced in the cell types found to express IL-17 mRNA *in vivo* (i.e., neutrophils and CD4⁺ and CD8⁺ cells). To do so, bronchoalveolar lavage neutrophils, obtained 1 day after the LPS challenge, and CD4⁺ and CD8⁺ cells purified from spleen were cultured and stimulated with either LPS or different concentrations of IL-15 for 24 and 48 h. In all the cell types, no IL-17 could be detected after 24-h stimulation. At 48 h, in both LPS- and IL-15-stimulated supernatant of neutrophils, IL-17 was detected. However, by that time, none of the cells had survived (not shown). At the same time point, IL-15 induced a dose-dependent production of IL-17 from CD4⁺ cells. In contrast, no IL-17 could be detected in the supernatants obtained from CD8⁺ cells (Fig. 7B). Neither CD4⁺ nor CD8⁺ cells produced IL-17 following stimulation with LPS (Fig. 7B).

Discussion

The present study was designed to explore the role of IL-17 in LPS-induced lung neutrophil infiltration. We have shown that IL-17, produced mainly by CD4⁺ cells, but also by neutrophils, drives the late phase neutrophilia following LPS challenge.

Endotoxin instillation into the lungs of BALB/c mice resulted in a reproducible inflammation in bronchoalveolar lavage fluids that occurs in two phases. The early phase from 1 to 24 h was characterized by a massive neutrophil influx, a small increase in lymphocytes, and a decrease in macrophage numbers. Production of cytokines TNF- α and IL-1 β and chemokines KC and MIP-2 was also evidenced. In the later phase from 24 h, neutrophil numbers started to decrease before increasing again and peaking at day 2. This was associated with a massive increase in macrophage and

lymphocyte numbers together with production of IL-17. During this later phase, KC, MIP-2, and TNF- α returned to basal levels, whereas IL-1 β production was sustained up to day 2.

TNF- α and IL-1 β have been previously shown to play a significant role in the lung neutrophilic infiltration. This study and the work of others (27–29) have shown that, following LPS provocation, a substantial release of both cytokines is observed. It was also demonstrated that lung-specific overexpression of these cytokines (30, 31) or direct application into the lung (27, 32) resulted in a massive influx of neutrophils. Moreover, the early lung neutrophil influx observed following bacterial challenge was partially blocked using neutralizing anti-TNF- α Abs in mice (27) or using an IL-1R antagonist in rat (33). KC and MIP-2, two powerful neutrophil chemoattractants, are also produced in the lung following LPS challenge (34, 35), and neutralization experiments have shown that these two chemokines play an important role in the lung neutrophil influx (35, 36). Although we did not perform neutralization experiments for all these mediators, the published data quoted above and their kinetics of production observed in our model suggest that these chemokines/cytokines are responsible for the early neutrophil influx.

IL-17 has been shown to induce recruitment of neutrophils into the rat airways via the production of C-X-C chemokines (12). This was confirmed in mice, using overexpression of lung IL-17 through adenovirus-mediated gene transfer (19) or using IL-17R-deficient mice following airway bacterial infection (18). In agreement with these data, we have shown that intranasal administration of IL-17 in BALB/c mice induced a neutrophil influx associated with a lung production of MIP-2 and KC. Moreover, the inhibition of the late phase neutrophilia, following LPS challenge, by the anti-IL-17-neutralizing Ab was associated with an inhibition of KC and MIP-2 production in the lungs.

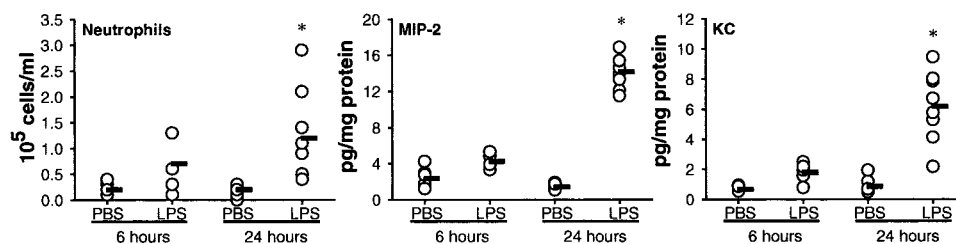


FIGURE 4. IL-17-induced bronchoalveolar lavage neutrophilia and lung chemokine production. Mice were intranasally challenged with 1 μ g of IL-17 or PBS. Bronchoalveolar lavage was performed and lungs were processed for chemokine measurement 6 or 24 h after the challenge. Individual data, from five to eight mice per group, and the mean (horizontal bar) are shown. Significance, indicated by *, is vs PBS-challenged animals at the same time point.

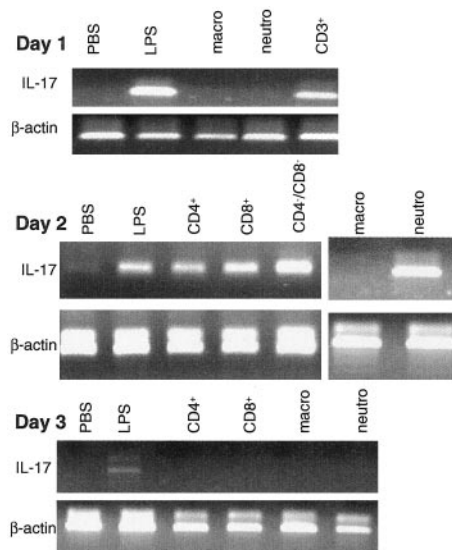


FIGURE 5. Time course of IL-17 mRNA expression by RT-PCR. Mice were intranasally challenged with LPS ($n = 20$) or PBS ($n = 10$) and sacrificed 1, 2, or 3 days after. Bronchoalveolar lavages were pooled, and cells from the LPS-challenged animals were purified by cell sorting and adherence to plastic, into lymphocyte subsets, neutrophils (neutro), and macrophages (macro). β -Actin was used as a housekeeping gene control.

In our model, IL-17 was not produced during the early inflammatory phase, but its peak production coincided with the second wave of neutrophil influx. Based on this, we hypothesized that endogenously produced IL-17, in response to LPS, was responsible for the late phase neutrophilia. Indeed, BALB/c mice treated with a neutralizing anti-IL-17 Ab were unable to develop a late neutrophil influx. Moreover, SCID mice, who develop a comparable early phase neutrophilia to BALB/c, did not develop a late phase neutrophilia, and this was associated with lower levels of IL-17. Interestingly, during the late phase inflammation, the levels of KC and MIP-2 in SCID mice were much lower when compared with BALB/c. This was probably a reflection of the lower levels of IL-17 produced in these animals due to lack of lymphocytes. Indeed, when treated with the neutralizing anti-IL-17 Ab, BALB/c mice produce far less KC and MIP-2. All together these data suggest that, in our model, the late phase neutrophilia is primarily driven by IL-17, through the production of KC and MIP-2.

The reason that IL-17 was produced only during the late phase neutrophilia is not clear. Both neutrophils and lymphocytes, which

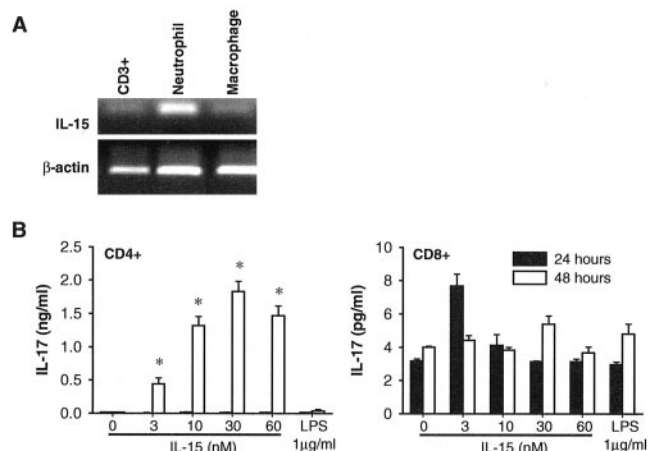
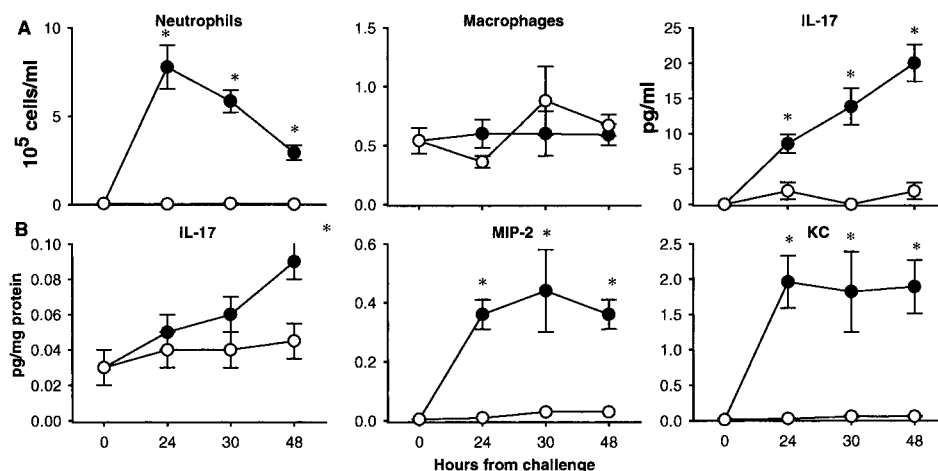


FIGURE 7. RT-PCR (A) analysis of IL-15 expression 1 day after LPS challenge. Mice ($n = 20$) were intranasally challenged with LPS and sacrificed 1 day after the LPS challenge. Bronchoalveolar lavage was pooled and purified by cell sorting and adherence to plastic, into CD3⁺, neutrophils, and macrophages. β -Actin was used as a housekeeping gene control. B, Effect of IL-15 and LPS on IL-17 production of CD4⁺ and CD8⁺ cells in vitro. Spleen CD4⁺ and CD8⁺ cells were cultured in the presence or absence of LPS or different concentrations of IL-15. Cell supernatants were collected after 1 or 2 days and analyzed for IL-17 production. Data are expressed as mean \pm SEM of triplicate measurements. Significance, indicated by *, is vs control cells.

produce IL-17 mRNA during the late phase response, were present in the bronchoalveolar lavage fluids before any IL-17 could be detected (from 3 to 24 h), suggesting that despite the presence of both cell types in this early inflammatory phase, an activation signal is missing. In this context, IL-15 was a likely candidate, because this cytokine, which can be produced in the airways by inflammatory cells such as macrophages and neutrophils (37), has been shown to be a potent trigger that leads to production of IL-17 (26). More importantly, IL-15 has been shown to activate both T cells (38) and neutrophils (39). Because of the lack of available tools, we could not measure IL-15 at the protein level or perform a neutralizing experiment. However, IL-15 was detected at the mRNA level, 24 h after the LPS challenge, and this expression was associated mainly with neutrophils. Our hypothesis was further supported by the fact that when stimulated in vitro with IL-15, CD4⁺ cells were able to release IL-17. Although indirect, these data suggest that in our model, IL-17 production could be triggered

FIGURE 6. LPS-induced inflammation in the bronchoalveolar lavage fluid (A) and in the lung (B) of SCID mice. Mice were intranasally challenged with LPS or PBS and sacrificed at different time points. Data are expressed as mean \pm SEM of two different experiments, and each included three to eight mice per group. Significance, indicated by *, is vs PBS-challenged animals at the same time point.



by an early release of IL-15 that is probably produced by neutrophils.

A recently described cytokine, IL-23, has been reported to regulate LPS-dependent IL-17 production from mononuclear splenocytes in vitro (40). Given that this observation was published during the preparation of this manuscript, we did not address the role of IL-23 in our model. However, one cannot exclude that IL-23 could also be produced following bacterial product challenge such as LPS in vivo, and thus regulates IL-17 production.

Of the large number of cell types analyzed for IL-17 expression, the CD4⁺ T cell has been previously reported to be the only producer of IL-17 (2–4, 9). In agreement with these observations, we report that CD4⁺ cells isolated from the bronchoalveolar lavage of BALB/c mice challenged with LPS expressed IL-17 at the mRNA level. However, we also found expression of IL-17 RNA in CD8⁺ cells and neutrophils. Although IL-17 mRNA expression by activated human CD8⁺ T cells has previously been shown (41), we are not aware of any data showing that this cell type is able to release IL-17 protein. In fact, our in vitro data suggest that despite the presence of the mRNA, murine CD8⁺ cells cannot release IL-17 when stimulated with IL-15. To our knowledge, this is the first demonstration that neutrophils are also able to produce IL-17 at the mRNA levels. Our in vitro data suggest that neutrophils are also able to produce IL-17 following stimulation with IL-15. However, IL-17 was detectable only after 2 days in culture. By that time, none of the neutrophils had survived, suggesting that IL-17 production either was associated with cell death or due to the contaminating lymphocyte population. Nevertheless, the demonstration that SCID mice, despite the lack of T cells, were also able to produce IL-17 in response to LPS challenge, associated with the observation that, in BALB/c mice, macrophages do not produce IL-17 mRNA, supports the fact that neutrophils have the capacity to produce this cytokine.

In summary, our results demonstrate that IL-17 is endogenously produced into the lung of mice following LPS challenge, and suggest that IL-15 is a possible trigger for this production. IL-17, produced mainly by CD4⁺ cells, but also by neutrophils, is responsible for the late phase neutrophilic infiltration, and this proinflammatory effect is associated with increased production of KC and MIP-2 into the lung tissue.

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