Role of IL-18 in CD4+ T Lymphocyte Activation in Sarcoidosis

Catherine M. Greene, Gerard Meachery, Clifford C. Taggart, Cyril P. Rooney, Raymond Coakley, Shane J. O’Neill and Noel G. McElvaney

J Immunol 2000; 165:4718-4724; doi: 10.4049/jimmunol.165.8.4718
http://www.jimmunol.org/content/165/8/4718

References
This article cites 57 articles, 19 of which you can access for free at:
http://www.jimmunol.org/content/165/8/4718.full#ref-list-1

Why The JI? Submit online.
• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Fast Publication! 4 weeks from acceptance to publication

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Role of IL-18 in CD4⁺ T Lymphocyte Activation in Sarcoidosis

Catherine M. Greene, Gerard Meachery, Clifford C. Taggart, Cyril P. Rooney, Raymond Coakley, Shane J. O'Neill, and Noel G. McElvaney

Sarcoidosis is a granulomatous disease of unknown etiology associated with the expansion of IL-2-producing activated CD4⁺ T lymphocytes. A number of factors including the recently described IL-18 have been implicated in IL-2 expression in vitro. We investigated the role of IL-18 in IL-2 expression in sarcoidosis. Eighteen individuals with sarcoidosis and 15 normal controls were studied. IL-18R expression and epithelial lining fluid (ELF) concentrations of IL-18 were significantly elevated in the sarcoid group (p = 0.0143 and 0.0024, respectively). Both AP1 and NF-κB, transcription factors that regulate IL-2 gene expression, were activated in vivo in sarcoid pulmonary CD4⁺ T lymphocytes. Transcription factor activity was not detected in pulmonary CD4⁺ T lymphocytes from normal controls or from peripheral blood CD4⁺ T lymphocytes from individuals with sarcoidosis, further evidence of compartmentalization of the lymphoproliferative process in this condition. We examined the effects of IL-18 on AP1 and NF-κB in Jurkat T cells in vitro. These effects were both time and dose dependent. Examination of transcription factor activation and IL-2 gene expression in Jurkat T cells revealed that sarcoid but not normal ELF activated AP1 and NF-κB, induced IL-2 gene transcription, and up-regulated IL-2 protein production. Addition of IL-18 to normal ELF also induced IL-2 mRNA accumulation, whereas correspondent depletion of IL-18 from sarcoid ELF using neutralizing Abs abrogated all of the effects. These data strongly implicate IL-18 in the pathogenesis of sarcoidosis via activation of AP1 and NF-κB, leading to enhanced IL-2 gene expression and IL-2 protein production and concomitant T cell activation. The Journal of Immunology, 2000, 165: 4718–4724.

Although the etiology of sarcoidosis remains unknown, the current consensus regarding its pathogenesis is that it results from exposure of genetically susceptible hosts to particular environmental factors (1). Individuals of all races and both sexes can be affected by the disorder which does, however, have a proclivity for individuals of certain ethnic or racial groups under the age of 40 years (1, 2). Cumulative incidence estimates predict a lifetime risk of sarcoidosis of 0.85 and 2.4% for U.S. whites and blacks, respectively (3). Familial clustering of the disease has provided evidence that genetic predisposition may be an important risk factor, with loci that influence T cell function, regulation of Ag recognition, and processing or regulation of matrix deposition and granuloma formation representing the most likely candidate genes (4, 5). In particular, individuals with specific HLA-DR genotypes appear to be at higher risk (6–10). To date, numerous environmental factors including transmissible agents, infectious organisms, and noninfectious environmental agents have been proposed as potential etiological agents responsible for sarcoidosis; however, the causative agent has yet to be definitively identified (11–16).

The pathological hallmarks of sarcoidosis are noncaseating granulomas. Lesions can occur in many organs; however, 90% of patients have pulmonary manifestations (17, 18). The condition is further characterized by the accumulation and expansion in affected organs of immunocompetent Th1 lymphocytes, principally of the CD4⁺ phenotype, that release IL-2 and IFN-γ (19–22). The immunological changes associated with pulmonary sarcoidosis likely result from this expanded pool of CD4⁺ T lymphocytes (19, 23–25).

IL-18, a recently discovered cytokine, has been identified as having a role in the Th1 response (26–28). IL-18 is primarily a monocyte/macrophage-derived cytokine (29). IL-18 transcripts have also been detected in a wide variety of different cell types, including fibroblasts, T and B cell lineages, and airway epithelium (27, 30, 31). IL-18 is expressed as a procytokine and is cleaved intracellularly by caspase-1 to a mature, biologically active form (32–35). It has recently been shown by immunocytochemistry that airway columnar epithelial cells express IL-18, with higher immunoreactivity evident in biopsies from individuals with sarcoidosis than in those from asthmatics (31); however, it was not determined whether this IL-18 has biological activity.

The role of IL-18 in T lymphocyte IFN-γ protein production has been elucidated. IL-18 synergizes with IL-12 for enhanced IFN-γ production (36, 37). Independently, IL-18 can weakly induce IFN-γ production. However, more potent IFN-γ expression occurs in the presence of an IL-12 costimulus (38). Analysis of the signaling pathways by which IL-18 can induce transcriptional activity of the IFN-γ promoter in primary human CD4⁺ T cells has shown that IL-18 can induce IFN-γ promoter activity via activation of AP1 (38). IL-18 also enhances T cell proliferation in vivo. This occurs via an IL-2-dependent pathway (29, 39).

In this study, we investigated the role of IL-18 in regulation of IL-2 expression and T cell activation in pulmonary sarcoidosis and showed that IL-18 and its receptor are present at higher than normal levels in the lungs of individuals with sarcoidosis. We examined the effect of IL-18 on activation of transcription factors that regulate IL-2 gene expression and determined what effect the

Department of Respiratory Research, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland

Received for publication March 27, 2000. Accepted for publication July 24, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by The Higher Education Authority of Ireland, The Health Research Board of Ireland, and The Royal College of Surgeons in Ireland.

2 Address correspondence and reprint requests to Dr. Noel G. McElvaney, Department of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland. E-mail address: respres@iol.ie

Copyright © 2000 by The American Association of Immunologists

0022-1767/00/$02.00
IL-18 present in sarcoid epithelial lining fluid (ELF) has on IL-2 gene expression in T cells. Our observations strongly implicate IL-18 in the pathogenesis of pulmonary sarcoidosis and identify it as an important factor regulating expression of the IL-2 gene, possibly via modulation of transcription factor activity.

Materials and Methods

Study population

Eighteen newly diagnosed, untreated, biopsy-proven individuals with stage I or stage II sarcoidosis and 15 normal healthy controls were evaluated for the study (male-to-female ratio, 9:9 and 6:9; mean age ± SEM 39.3 ± 2.4 and 56.5 ± 3.7, respectively). Pulmonary function tests results (percent predicted mean ± SEM) for the sarcoid and control group did not differ significantly. Forced expiratory volume in 1 s, 86 ± 8 and 89 ± 6; forced expiratory volume in 1 s/forced vital capacity, 77 ± 9 and 74 ± 12; diffusion capacity of carbon monoxide, 72 ± 13 and 79 ± 10, respectively).

Measurement of ELF cytokine and caspase-1 levels

CD4 T lymphocytes were rosetted by placing each sample in a magnetic particle isolation column (Dynal, Wirral, U.K.) for 4 h at 4°C with rotation. CD4 T lymphocytes caspase-1 levels in unconcentrated BAL fluid samples were measured by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA). Briefly, T lymphocytes had been obtained. A similar protocol was used, except for cytology. The volume of BAL fluid recovered in different disease states may vary; to standardize this, we quantified the volume of ELF recovered by lavage using the urea dilution method (Sigma, Poole, U.K.). These were 1.0 ± 0.17 ml for the sarcoid group and 0.9 ± 0.12 ml for the normal group (mean ± SEM) (41). An inflammatory control group of 17 acute pneumonia patients was also used for measurement of IL-18 ELF levels and IL-18R expression. ELF recovery was 0.7 ± 0.12 ml.

Measurement of ELF cytokine and caspase-1 levels

CD4 T lymphocytes from ELF and peripheral blood

After washing with 30 ml HBSS (Life Technologies), the BAL cell pellet was resuspended in 1 ml RPMI 1640 containing 10% FCS (Life Technologies). The BAL fluid cells were incubated with 1 × 106 washed M-450 CD4 Dynabeads (Dynal, Wirral, U.K.) for 1 h at 4°C with rotation. CD4 T lymphocytes were rosetted by placing each sample in a magnetic particle concentrator for 2 min. The cells were washed five times with HBSS and then resuspended in 100 µl medium. The Dynabeads were removed from the CD4+ cells by incubation with 10 µl DETACHaBEAD CD4/CD8 (Dynal) for 30 min at room temperature with rotation. After rosetting of the Dynabeads, cell size and granularity, as indicated by low-angle forward and side scattering properties of argon laser light (488 nm), were assessed by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA).

Isolation of CD4+ T lymphocytes from ELF and peripheral blood

After washing with 30 ml HBSS (Life Technologies), the BAL cell pellet was resuspended in 1 ml RPMI 1640 containing 10% FCS (Life Technologies). The BAL fluid cells were incubated with 1 × 106 washed M-450 CD4 Dynabeads (Dynal, Wirral, U.K.) for 1 h at 4°C with rotation. CD4 T lymphocytes were rosetted by placing each sample in a magnetic particle concentrator for 2 min. The cells were washed five times with HBSS and then resuspended in 100 µl medium. The Dynabeads were removed from the CD4+ cells by incubation with 10 µl DETACHaBEAD CD4/CD8 (Dynal) for 30 min at room temperature with rotation. After rosetting of the Dynabeads, cell size and granularity, as indicated by low-angle forward and side scattering properties of argon laser light (488 nm), were assessed by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA).

Caspase-1 expression

Caspase-1 cleaves pro-IL-18 to its mature and active form; measurement of IL-18 present in sarcoid epithelial lining fluid (ELF) has on IL-2 expression was performed. CD4+ T lymphocytes were centrifuged at 500 × g for 10 min. The supernatant was aliquoted and stored at −80°C for further evaluation. The cell pellet was resuspended in 1 ml RPMI 1640 containing 10% FCS (Life Technologies, Paisley, U.K.). Cells were counted in a hemocytometer, and cytospin slides were prepared for cytology. The volume of BAL fluid recovered in different disease states may vary; to standardize this, we quantified the volume of ELF recovered by lavage using the urea dilution method (Sigma, Poole, U.K.). These were 1.0 ± 0.17 ml for the sarcoid group and 0.9 ± 0.12 ml for the normal group (mean ± SEM) (41). An inflammatory control group of 17 acute pneumonia patients was also used for measurement of IL-18 ELF levels and IL-18R expression. ELF recovery was 0.7 ± 0.12 ml.

Preparation of nuclear extracts

Nuclear extracts were isolated from CD4+ T lymphocytes and Jurkat cells. Briefly, cells were washed with 5 ml ice-cold PBS and resuspended in 1 ml hypotonic buffer (10 mM HEPES (pH 7.9), 1.5 mM MgCl2, 10 mM KCl, 0.5 mM PMSF, and 0.5 mM DTT) (Sigma). Cells were centrifuged at 500 × g for 15 min, washed three times with isotonic PBS (Life Technologies) supplemented with 0.5% BSA (Sigma), and resuspended in the same buffer at 4 × 106 cells/ml. Cells (1 × 106; 25 µl) were Fc blocked by treatment with 1 µg human IgG for 15 min at room temperature. The cells were then stained directly with 10 µl monoclonal anti-human PE-labeled anti-IL-18R Ab or a mouse PE-conjugated IgGl isotype control Ab (R&D Systems) for 45 min at 4°C. Cells were washed as before and then analyzed by flow cytometry (FACScan), measuring fluorescence emission at >610 nm.

To quantify IL-18 expression on sarcoid, normal, and inflammatory control BAL CD4+ T cells, cytospin preparations were blocked as described and stained with 10 µl each of monoclonal anti-human PE-C55-labeled anti-CD4 Ab and PE-labeled anti-anti-IL-18 Ab or PE-conjugated IgGl isotype control Ab (R&D Systems). Cellular fluorescence of at least 5 × 105 cells was measured by laser scanning cytometry. Fluorescence excitation was provided by a 488 nm laser line. Far-red fluorescence (PE-CYS) and red fluorescence (PE) were measured at >650 and >610 nm, respectively. The threshold contour was set on far-red fluorescence to detect all PE-C55-labeled CD4+ T cells. Artificially contoured debris was gated out based on contour size. Individual IL-18R-positive CD4+ T cells were then identified and quantified using CompuCyte software on the basis of integrated red fluorescence, reflecting binding of the anti-IL-18R-PE Ab.

IL-2 mRNA analysis

Total RNA was isolated from 1 × 106 CD4+ T lymphocytes (isolated by peritoneal washout for an additional 24 h) and lysed for 10 min in a lysis buffer (10 mM HEPES (pH 7.9), 0.5 mM EDTA, 25% (v/v) glycerol, 0.5 mM PMSF) (Sigma) for 15 min on ice. After centrifugation at 13,000 × g for 15 min at 4°C, nuclear extracts were removed into 50 µl storage buffer (10 mM HEPES (pH 7.9), 50 mM KCl, 0.2 mM EDTA, 20% (v/v) glycerol, 0.5 mM PMSF, and 0.5 mM DTT). Protein concentrations were determined (43), and the extracts were stored at −80°C.

EMA

Nuclear extracts (4 µg from Jurkat cells, 1 µg from CD4+ T lymphocytes) were incubated with 10,000 cpm of [γ-32P]ATP (Amersham Pharmacia Biotech, Little Chalfont, U.K.) T4 polynucleotide kinase (Promega, Madison, WI) end-labeled oligonucleotides containing AP1, NF-xB, or NF-AT consensus sequences (Santa Cruz Biotechnology, Santa Cruz, CA). Incubations were performed for 30 min at room temperature in binding buffer (4% (v/v) glycerol, 1 mM EDTA, 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM DTT, 0.1 mg/ml nuclease-free BSA) and 2 µg poly(dI-dC)·poly(dI-dC).poly(dI-dC)·poly(dI-dC) (Sigma). In some experiments, unlabeled wild-type or mutant oligonucleotides (Santa Cruz Biotechnology) were added to the extracts before incubation with the labeled oligonucleotide. Reaction mixtures were electrophoresed on native 5% polyacrylamide gels that were subsequently dried and autoradiographed.

Abbreviations used in this paper: ELF, epithelial lining fluid; BAL, bronchoalveolar lavage; ICS, internal calibration standard.

The Journal of Immunology

4719
ELF that had been depleted of IL-18. Mouse anti-human IL-18 IgG2a-neutralizing Abs (R&D Systems) were added to sarcoid BAL fluid to a final concentration of 1 μg/ml for 1 h at room temperature. The Ab-Ag complexes were then removed by immunoprecipitation with protein A-agarose (30 μl) for 2 h at 4°C (Roche Biochemicals, Lewes, U.K.). Protein A beads and Ab-antigen complexes were removed by centrifugation at 13,000 × g for 2 min. Control experiments using an isotype control mouse IgG2a Ab (final concentration, 1 μg/ml) were also performed (R&D Systems).

For competitive IL-2 PCR, 1 μg total RNA was reverse transcribed into cDNA with an oligo(dT) 15 primer, using first strand cDNA synthesis kit (Roche Biochemicals) according to the manufacturer’s instructions. The integrity of RNA extraction and cDNA synthesis was verified by PCR by measuring the amount of GAPDH cDNA in each sample using GAPDH-specific primers (BioSource International, Camarillo, CA). PCR reaction mixtures contained 10 mM KCl, 10 mM (NH₄)₂SO₄, 20 mM Tris-HCl (pH 8.8), 2 mM MgSO₄, 0.1% Triton X-100, 2.5 U Taq polymerase, and 2 mM each dNTP (Promega, Madison, WI). Thermocycling conditions for IL-2 cDNA were 95°C for 2.5 min, 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Fifteen cycles were used to amplify the more abundant GAPDH cDNA. A final extension step of 72°C for 10 min was followed by resolution of the 382-bp GAPDH products on a 1.5% triethylammonium agarose gel (Roche Biochemicals) containing 0.5 μg/ml ethidium bromide (Sigma). Conditions for quantitative competitive PCR (CytoXpress quantitative PCR kit for human IL-2, BioSource International) were determined by carrying out titration curves for IL-2 cDNAs and an IL-2 internal calibration standard (ICS) as described in the manufacturer’s protocol. The ICS template contained PCR primer-binding sites identical with those of the IL-2 cDNA, which generated a 412-bp product after PCR amplification. This was easily distinguishable by agarose gel electrophoresis from the 382-bp IL-2 PCR product. ICS and IL-2 PCR products were quantified by densitometry using GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, U.K.) and Syngene GeneSnap and GeneTools software.

IL-2 protein production by Jurkat T cells

IL-2 was measured in supernatants of cells stimulated with PMA (50 ng/ml, Sigma) plus A23187 (500 ng/ml) and IL-18 (30 ng/ml) or 10 μl normal or sarcoid ELF, or sarcoid ELF that had been depleted of IL-18, as described above, for 24 h by ELISA. To prevent the IL-2 present in sarcoid ELF interfering with the assay, sarcoid ELF was depleted of IL-2 using mouse anti-human IL-2 IgG2a-neutralizing Abs (R&D Systems), 1 μg/ml BAL as described for IL-18, before addition to the cells. Control experiments using an isotype control mouse IgG2a Ab (final concentration, 1 μg/ml) were also conducted (R&D Systems).

Statistical analysis

Data were analyzed with the GraphPad Prism 2.0 software package (GraphPad Software, San Diego, CA). Results are expressed as mean ± SEM and were compared by Mann-Whitney and Pearson r value correlation.

Results

Th1 cytokine and IL-18 levels in normal and sarcoid ELF

Sarcoid ELF had a higher lymphocyte-macrophage ratio than did normal ELF (1:5 vs 1:15), consistent with pulmonary CD4⁺ T lymphocyte expansion, a characteristic of the condition (21, 22). Consistent with previous published data (19, 20), IL-2 concentrations were significantly elevated in sarcoid ELF compared with the normal group with values of 2717 ± 1130 vs 1319 ± 561 pM ELF, respectively (p = 0.0211). In addition to IL-2, a classical Th1 cytokine, both IL-12 p40 and IFN-γ levels were also significantly elevated in the sarcoid group compared with normals (24, 44, 45). Mean levels of both cytokines were 3-fold higher in sarcoid ELF: 331 ± 112 vs 99 ± 20 pM (p = 0.0013) for IL-12 p40 and 6302 ± 2682 vs 1891 ± 1298 pM (p = 0.025) for IFN-γ.

Sarcoid IL-18 levels (5301 ± 2148 pM ELF) were significantly higher than those of both the normal group (816 ± 650 pM ELF, p = 0.0024) and an inflammatory control group (536 ± 96 pM ELF, p = 0.0416). A positive correlation was observed between IL-2 and IL-18 sarcoid ELF levels (r = 0.8984, p < 0.0001). There was no difference in caspase-1 levels in ELF from individuals with sarcoidosis and normal controls.

IL-18R expression by Jurkat T cells, peripheral blood, and BAL CD4⁺ T lymphocytes

Flow cytometric analysis using a PE-labeled anti-IL-18R mAb or a mouse PE-conjugated IgG1 isotype control Ab revealed that Jurkat T cells constitutively express IL-18R (Fig. 2A). Mean channel fluorescence detected at >610 nm, was significantly higher for anti-IL-18R than for isotype control Ab-labeled Jurkat cells (Fig. 2A). As a control, myelomonocytic U-937 cells were also analyzed. These cells are known to constitutively express IL-18R (42) (Fig. 2A). Normal peripheral blood CD4⁺ T cells expressed low concentrations of IL-18R. IL-12 stimulation up-regulated IL-18R expression on these cells (Fig. 2B). Sarcoid BAL CD4⁺ T cells expressed IL-18R at concentrations significantly elevated compared with normal (p = 0.0143) and inflammatory control (p = 0.0286) CD4⁺ BAL T cells (Fig. 2C).

API

Activation by IL-18 in vitro. The IL-2 gene promoter carries response elements for a number of transcription factors including NF-AT and NF-κB; however, binding of API to its recognition site is essential for IL-2 expression (46). Fig. 3 shows that IL-18 activated API in Jurkat T cells in a dose- and time-dependent manner with optimal activation evident at 30 ng/ml IL-18 (Fig. 3A) for 2 h (Fig. 3B). DNA-protein interactions were inhibited by pre-incubation with unlabeled oligonucleotide containing a wild-type but not a mutated AP1-binding site (data not shown). IL-18 had no effect on NF-AT activation in Jurkat T cells (data not shown).

Activation in vivo in sarcoid pulmonary but not peripheral blood CD4⁺ T lymphocytes. Active API complexes were present in the nuclei of the sarcoid CD4⁺ T lymphocytes isolated from ELF (Fig. 3C). Levels of IL-18 in sarcoid ELF are 5301 ± 2148 pM corresponding to 10.7 ± 4.3 ng/ml BAL, which is a physiologically relevant concentration. In contrast, API DNA binding activity was not detected in peripheral blood T lymphocytes isolated from all of the same individuals, suggesting a compartmentalized response, localized to the lungs (Fig. 3C).

NF-κB

Activation by IL-18 in vitro. Time-course and dose-response experiments to examine the effect of IL-18 on NF-κB activation in Jurkat T cells confirmed previous reports in other cells, with optimal activation induced at 30 min by 30 ng/ml IL-18 (data not shown) (47–51).
Effect of IL-18 in ELF on IL-2 gene expression and IL-2 protein production

IL-18 induced IL-2 gene expression in TCR-activated Jurkat T cells (Fig. 6, lane 2). Indeed, IL-18 could also potentiate PMA/A23187-induced IL-2 production from Jurkat cells by 27 ± 1.1% (2047 ± 63 vs 1502 ± 23 pg/ml IL-2, respectively). Incubation of Jurkat T cells with normal ELF (Fig. 6, lane 3) had no effect on IL-2 mRNA accumulation without addition of exogenous IL-18 (lane 4). In contrast, sarcoid ELF alone strongly activated the IL-2 promoter, an effect that was abrogated by depletion of IL-18 from sarcoid ELF using an anti-IL-18-neutralizing Ab and immunoprecipitation (Fig. 6, lanes 5 and 6). An isotype control Ab had no effect (Fig. 6, lane 7). Similar to these IL-2 mRNA assays, concentrations of IL-2 protein produced from cells treated with normal ELF or sarcoid ELF depleted of IL-18, using a neutralizing Ab, were 66 + 4.3% less than IL-2 concentrations produced in response to stimulation with sarcoid ELF.

Effect in vivo in sarcoid pulmonary CD4+ T lymphocytes.

Investigation of the status of NF-κB activity in sarcoid pulmonary CD4+ T lymphocytes indicated that NF-κB was activated and localized to the nucleus of these cells (Fig. 4). Correspondingly, similar experiments with normal respiratory ELF CD4+ T lymphocytes failed to detect any NF-κB DNA binding activity in the nuclear extracts (Fig. 4).

Effect of IL-18 in ELF on transcription factor activation

Stimulation of Jurkat T cells with 10 μl sarcoid, but not normal, ELF for 2 h activated AP1 (Fig. 5A, lanes 2 and J, respectively). Depletion of IL-18 from the sarcoid ELF using an anti-IL-18-neutralizing Ab and immunoprecipitation abrogated this effect (lane 3). An isotype control Ab had no effect (Fig. 5, lane 4). Similarly, as shown in Fig. 5B, NF-κB was activated by sarcoid ELF, after a 30-min stimulation (Fig. 5, lane 2). However, normal ELF, or sarcoid ELF from which the IL-18 had been neutralized, could not induce NF-κB activation (Fig. 5, lanes 1 and 3). Lane 4 shows the isotype control.

Effect of IL-18 in ELF on IL-2 gene expression and IL-2 protein production

FIGURE 3. AP1 activation in Jurkat T cells and sarcoid peripheral blood and pulmonary CD4+ T lymphocytes. AP1 activation was measured by EMSA using a [γ32P]ATP end-labeled AP1 consensus sequence (10,000 cpm) in nuclear extracts (4 μg) from control Jurkat T cells and cells stimulated with 10 or 30 ng/ml IL-18 for 2 h (A) and in nuclear extracts (4 μg) from control Jurkat T cells and cells stimulated with 30 ng/ml IL-18 for 30 min, 1 h, or 2 h (data representative of three separate experiments) (B) and in nuclear extracts (1 μg) from sarcoid peripheral blood and respiratory epithelial lining fluid (ELF) CD4+ T lymphocytes, isolated using anti-CD4-coated magnetic beads (C). E = ELF CD4+ T cells, B = peripheral blood CD4+ T cells (n = 9). The results are shown for two patients (#1 and #2). Arrows, AP1 DNA-protein complexes.

FIGURE 4. NF-κB activation in normal and sarcoid pulmonary CD4+ T lymphocytes. CD4+ T lymphocytes were isolated from normal and sarcoid ELF as described, and nuclear extracts were prepared. Reaction mixtures containing 1 μg nuclear protein and 10,000 cpm [γ32P]ATP end-labeled NF-κB consensus sequence were resolved by electrophoresis on a 5% polyacrylamide gel. Arrows, NF-κB DNA-protein complexes. N = normal, S = sarcoid (n = 11). Two representative results are shown for each.
FIGURE 5. Effect of normal and sarcoid ELF on transcription factor activation in Jurkat T cells. AP1 (A) and NF-κB (B) activation were measured by EMSA using [γ-32P]ATP end-labeled consensus sequences (10,000 cpm) in nuclear extracts (4 μg) from Jurkat T cells stimulated with normal ELF (lane 1), sarcoid ELF (lane 2), sarcoid ELF depleted of IL-18 (lane 3), and sarcoid ELF isotype control (lane 4) for 2 h (A) and 30 min (B) (n = 5). Arrows, AP1 and NF-κB DNA-protein complexes.

Discussion

This study shows that IL-18 has an important role in the regulation of IL-2 expression by pulmonary CD4+ T lymphocytes in sarcoidosis. ELF concentrations of IL-18 and IL-18R expression were significantly elevated in individuals with sarcoidosis. The transcription factors AP1 and NF-κB were active in vivo in sarcoid CD4+ T lymphocytes, a scenario that was reproduced in vitro by stimulation of Jurkat T cells with IL-18 or sarcoaid ELF. Sarcoaid, but not normal, ELF also induced IL-2 promoter activation in CD3-stimulated Jurkat T cells and depletion of IL-18 from sarcoaid ELF abrogated this effect. Furthermore, supplementation of normal ELF with IL-18 induced IL-2 mRNA accumulation. The proinflammatory role of IL-18 in sarcoaid ELF was demonstrated by its potentiation of IL-2 protein production compared with normal ELF or IL-18-depleted sarcoaid ELF. These findings further establish IL-18 as an important factor in IL-2 gene expression and thus in the pathogenesis of sarcoidosis via modulation of regulatory transcription factor activity.

Sarcoaidosis is a chronic granulomatous disease of unknown etiology. It is a multisystem disorder that primarily affects the lung and lymphatic systems but other organs including the liver, spleen, salivary glands, heart, nervous system, muscles, and bones may also be affected (2, 52). The characteristic granulomas associated with sarcoaidosis are discrete and noncaseating, consisting of lymphocytes, epithelioid cells, and giant cells. In addition to granuloma formation, pulmonary sarcoaid disease is characterized by the expansion of Th1 cells in the lung due to a cellular redistribution from the peripheral blood (19–22). This lymphocytosis is controlled by chemoattractant cytokines which cooperate to expand the intraalveolar pool of CD4+ T cells within the inflamed area (25, 53, 54). In situ IL-2-mediated proliferation further contributes to CD4+ T cell accumulation at sites of granuloma formation (19, 25, 55). There has been much speculation regarding what factors are responsible for the enhanced IL-2 expression of these CD4+ T cells. However, the immunological pattern of cells in sarcoaid infiltrates suggests that a poorly degradable persistent antigenic stimulus may be responsible for the induction of proinflammatory and chemotactic mediators in the lung, resulting in an acute and often chronic Th1 inflammatory response, which is characterized by elevated levels of IL-2 and IFN-γ.

IL-18 has an important role in the Th1 response (26–28). Although it is primarily a monocyte/macrophage-derived cytokine (29), IL-18 transcripts have also been detected in a variety of other cell types, including airway epithelium (27, 30, 31). Immune-reactive IL-18 has recently been shown to be present in airway columnar epithelial cells in biopsies from individuals with sarcoaidosis IL-18 (31), providing another potential source, in addition to macrophages, of the IL-18 present in ELF in sarcoaidosis. Although this suggests an important role for IL-18 in the pathogenesis of sarcoaidosis, it is also possible that IL-18, with its many proinflammatory properties, may play a role in other chronic pulmonary conditions. Importantly, in this study, the mature IL-18 in sarcoaid ELF displayed potent activity as shown by its many biological effects. Unlike IL-18, caspase-1 levels in sarcoaid ELF in this study were not elevated. However, this might not necessarily reflect intracellular concentrations of caspase-1 in alveolar macrophages.

IL-12 can synergize with IL-18 to enhance IFN-γ production (36–38). In addition to IL-18, levels of both IL-12 and IFN-γ were significantly elevated in ELF from our sarcoaid compared with normal populations, which supports this theory and confirms previous findings (24, 44, 45). IL-12 derived from APCs induces IL-18R expression on T lymphocytes, thereby priming the cells for IL-18 responsiveness. Here we demonstrated this effect on IL-18R expression by stimulating CD4+ T lymphocytes isolated from peripheral blood with IL-12.

We and others have shown that IL-18 can induce IL-2 production by Ag- or anti-CD3-stimulated T cells in vitro. Interestingly, these stimuli are unable to induce IL-18 responsiveness in peripheral blood CD4+ T lymphocytes due to the fact that resting T helper cells express only low concentrations of IL-18R (38). In our study, Jurkat T cells constitutively expressed the IL-18R and therefore displayed IL-18 responsiveness. This was clearly shown by their ability to activate AP1 and NF-κB, induce IL-2 mRNA accumulation, and potentiate IL-2 protein production, in response to IL-18. We have shown here, for the first time, that IL-18R expression was significantly higher on sarcoaid BAL CD4+ T cells than on normal and inflammatory control BAL cells. This suggests that in vivo sarcoaidosis IL-18R expression on activated pulmonary CD4+ T lymphocytes is likely regulated by IL-12, as reflected by the elevated sarcoaid IL-12 ELF levels found in this study.

FIGURE 6. Effect of IL-18 and normal and sarcoid ELF on IL-2 mRNA expression in Jurkat T cells. CD3-stimulated Jurkat T cells (1 × 10⁶) were left untreated or stimulated with IL-18 (30 ng/ml) or 10 μl ELF for 4 h at which point total RNA was extracted. RNA (1 μg) was reverse transcribed into cDNA and used as template in quantitative competitive PCR reactions containing a known quantity of exogenously synthesized ICS. Control reactions measured levels of GAPDH mRNA. Products were electrophoresed in 1.5% triethylammonium agarose gels containing 0.5 μg/ml ethidium bromide, visualized under UV light, and quantified by densitometry. Values are expressed as input copy number of IL-2 mRNA after normalization to GAPDH and ICS. Data are representative of five independent experiments. Lane 1, Control; Lane 2, IL-18 (30 ng/ml); Lane 3, normal ELF; Lane 4, normal ELF plus IL-18 (30 ng/ml); Lane 5, sarcoaid ELF; Lane 6, sarcoaid ELF depleted of IL-18; Lane 7, sarcoaid ELF isotype control.
DNA footprinting of IL-18-stimulated T cells has documented occupancy of an AP1-binding site in the IFN-γ promoter (37). Our data showing the effect of IL-18 on AP1 activation in Jurkat T cells and findings from our in vivo studies in sarcoid pulmonary CD4+ T lymphocytes support these observations and suggest that AP1 regulates not only the IFN-γ but also the IL-2 promoter in vivo. Although AP1 can cooperate with NF-AT, we found that IL-18 had no effect on NF-AT activation. This is perhaps not all too surprising considering that TCR/CD3 signaling controls NF-AT activation (56).

The importance of the transcription factor NF-κB in IL-2 gene regulation has been well documented (39, 46, 57). NF-κB is activated by a wide range of proinflammatory stimuli and recent reports have identified NF-κB inducibility as a property of IL-18 (31, 32). This study is the first report showing a role for IL-18 in NF-κB and AP1 activation and IL-2 gene expression in sarcoidosis. We have shown that NF-κB was activated and localized to the nucleus of sarcoid but not control respiratory ELF CD4+ T lymphocytes. Uniquely, this study has also shown that AP1 was activated in sarcoid respiratory ELF CD4+ T lymphocytes but not peripheral blood, presumably in response to proinflammatory signals, possibly IL-18.

Current therapy for sarcoidosis is aimed at suppressing the inflammatory response, reducing the burden of granulomas, and preventing the development of fibrosis. Corticosteroids are the most commonly used agents for the treatment of sarcoidosis and, because of their ability to attenuate the inflammatory response, are thought to be capable of slowing or halting the progression of fibrosis that can develop. Steroids regulate inflammatory gene expression via inhibition of NF-κB activity. The IL-18 promoter carries recognition sites for NF-κB (30) and as such represents a potential target for these drugs. Therapies based on modulation of IL-18 signaling events may have potential for the treatment of sarcoidosis. However, further studies to evaluate the implications of elevated IL-18 ELF levels will be required. It is thought that CD4+ T lymphocytes is a good prognostic indicator for pulmonary sarcoidosis. Our data show that IL-2 expression, an integral event in CD4+ T lymphocytes, is regulated by IL-18, thereby suggesting that increased IL-18 may not necessarily be acting in a deleterious manner. By inducing this type of acute inflammatory response, reducing the burden of granulomas, and possibly IL-18, sarcoidosis: a review and summary of recent molecular biological data. Sarcoidosis 12:20.

References


