Activator Protein-1 Activation in Acute Lung Injury

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The role of activator protein-1 (AP-1) in inflammation is primarily unknown. AP-1 was evaluated in nuclear extracts from alveolar macrophages and whole lung nuclear extracts during acute lung injury after deposition of IgG immune complexes. AP-1 activation occurred in macrophages and in whole lung extracts, but with distinctly different time courses. Low levels of constitutive AP-1 were observed in normal rat lung as determined by the electrophoretic mobility shift assay. Increased AP-1 was detected 2 hours after initiation of the inflammatory response in lung with a further increase by 4 hours, while AP-1 activation was found in alveolar macrophages 0.5 hour after onset of the inflammatory response. mRNAs and proteins for c-fos, c-jun, jun-B, and jun-D were all up-regulated in whole lung tissues and in alveolar macrophages during acute lung injury induced by IgG immune complex deposition. Depletion of lung macrophages sharply reduced AP-1 activation, as did anti-tumor necrosis factor- α , whereas complement depletion showed no effect on lung AP-1 activation. The data suggest that activation of AP-1 occurs in both alveolar macrophages and in the lung, and this activation process is macrophage- and tumor necrosis factor- α -dependent. (*Am J Pathol* 2002, 161:275–282)

The transcription factor, activator protein-1 (AP-1), which is comprised of a number of homodimeric and heterodimeric complexes of members of the jun family (c-jun, jun-B, and jun-D) and Fos (c-fos, fos-B, fra1, and fra2) family, is known to be involved in cell proliferation and related events. Activation of AP-1 is associated with increased transcription leading to increased expression of AP-1 proteins and posttranslational modifications (such as phosphorylation), which may either increase or decrease transactivation of the AP-1 complex.^{1,2} AP-1 activation occurs in response to a number of diverse stimuli, including oxidative or cellular stress, ultraviolet

irradiation, DNA damage, antigen binding by T or B lymphocytes, and exposure to proinflammatory cytokines [eg, tumor necrosis factor (TNF)- α , transforming growth factor- β , and γ interferon].³ Much of what is known about the biological function of AP-1 relates to its prominent roles in cell proliferation, differentiation, transformation, and apoptosis.^{1,3} Very little is known regarding the function of AP-1 in the inflammatory response. Interestingly, the promoter regions of many inflammatory cytokines and chemokines [including TNF- α , interleukin (IL)-1 β , IL-6, IL-8, RANTES, and MCP-1] contain AP-1-binding sites^{4–6} suggesting that AP-1 activation may be necessary for the induction of acute, cytokine-mediated inflammation.

Intrapulmonary deposition of IgG immune complexes in rats induces acute lung injury that is characterized by extensive accumulation of neutrophils, interstitial and intra-alveolar edema, and hemorrhage.⁷ The inflammatory pathways in this model are rather similar to those observed in acute lung injury resulting from sepsis, infection, hemorrhagic shock, and remote organ ischemia and depend on the production of numerous cytokines and chemokines, many of which are known to be controlled in part by AP-1.^{1,8} The expression of inflammatory mediators in this model seems to involve the activation of the transcription factor, nuclear factor (NF)- κ B.⁹ Numerous *in vitro* studies have demonstrated that gene expression for many inflammatory mediators requires transcriptional activation of both AP-1 and NF- κ B. Although our earlier work has defined the role of NF- κ B in acute lung injury, little is known regarding the function of AP-1 in the lung model of acute inflammation. A recent report has shown that AP-1 is activated in adult rat lungs after 3 days of hyperoxia,¹⁰ but the role of AP-1 in acute lung inflammatory injury is unknown. In the current studies, we examined the temporal activation of AP-1 in alveolar macrophages and in whole lung tissues during IgG immune complex alveolitis. Our data demonstrate that activation of AP-1 occurs rapidly in alveolar macrophages and is then propagated to other lung cells.

Materials and Methods

Reagents

Liposomes composed of egg phosphatidylcholine and cholesterol and containing either phosphate-buffered sa-

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line (PBS), pH 7.4, or Cl₂MDP (dichloromethylene diphosphonate; a gift from Roche Diagnostics GmbH, Mannheim, Germany) were synthesized as described previously.¹¹ Rabbit polyclonal IgG anti-bovine serum albumin (BSA) was purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). Recombinant murine TNF- α was purchased from R&D Systems, Inc. (Minneapolis, MN). Rabbit polyclonal IgG anti-murine TNF- α , which is reactive with rat TNF- α , was produced and purified as previously described.¹² Unless otherwise specified all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

IgG Immune Complex-Induced Alveolitis in Rats

Pathogen-free male Long-Evans rats (275 to 300 g) (Harlan Sprague-Dawley, Indianapolis, IN) were anesthetized with ketamine-HCl (150 mg/kg, intraperitoneally). Rabbit polyclonal IgG anti-BSA (2.5 mg) in a volume of 0.3 ml in PBS, pH 7.4, were instilled intratracheally during inspiration. Immediately after intratracheal instillation of anti-BSA, 10 mg of BSA in 0.5 ml of PBS were injected intravenously. Negative control rats received PBS, pH 7.4, intratracheally. Unless otherwise indicated, 4 hours after initiation of IgG immune complex deposition rats were exsanguinated, the pulmonary circulation flushed via the pulmonary artery with 10 ml of PBS, and the lungs surgically dissected. For analysis of AP-1, lungs were immediately frozen in liquid nitrogen after vascular perfusion with PBS. For intervention studies, 300 μ g of pre-immune IgG or anti-TNF- α IgG were instilled intratracheally at the initiation of lung injury.

Alveolar Macrophage and Complement Depletion Studies

Rats were anesthetized with ketamine-HCl (150 mg/kg, intraperitoneally). A suspension of Cl₂MDP-liposomes in PBS (100 μ l of the liposome stock in a total volume of 500 μ l) was administered intratracheally during inspiration. As a control, PBS-liposomes were administered in a similar manner. All subsequent interventions were performed 24 hours after liposome instillation. We have previously shown that rats receiving Cl₂MDP-liposomes have 74% less alveolar macrophages than rats receiving PBS-liposomes.¹³ Administration of PBS-liposomes did not reduce the number of alveolar macrophages. Complement depletion in rats was accomplished by intraperitoneal injection of 25 U of purified cobra venom factor at 36, 24, and 12 hours before the induction of IgG immune complex-induced lung injury. This methodology reduces plasma C3 levels to less than 3% of their original levels.¹⁴

Alveolar Macrophages

Alveolar macrophages were isolated by repeatedly lavaging lungs of control or IgG immune complex-injured rats at the time points indicated. Cells were collected by centrifugation of lavage fluids, and nuclear protein was

extracted as described below. Cells retrieved from rats 0.5 or 1 hour after pulmonary deposition of IgG immune complexes by bronchoalveolar lavage fluid (BAL) were predominately alveolar macrophages (> 95%), as determined by staining of BAL cells with Camco Quick Stain (Cambridge Diagnostic Products, Ft. Lauderdale, FL).

Assessment of AP-1 Activation by Electrophoretic Mobility Shift Assay (EMSA)

Nuclear extracts from alveolar macrophages were prepared using a nuclear extraction kit (Pierce Co., Rockford, IL) according to the manufacturer's instructions. Protein concentrations were determined by bicinchoninic acid assay with trichloroacetic acid precipitation using BSA as a reference standard (Pierce Co.). Double-stranded AP-1 consensus oligonucleotide (5'-CGCTTGATGAGTCAGCCGGAA-3'; Promega, Madison, WI) was end-labeled with γ -[³²P] ATP (3000 Ci/mmol at 10 mCi/ml; Amersham, Arlington Heights, IL). Binding reactions containing 10 μ g of nuclear extracts and 35 fmol (~50,000 cpm, Cherenkov counting) of oligonucleotide were performed for 30 minutes in binding buffer (4% glycerol, 1 mmol/L MgCl₂, 0.5 mmol/L ethylenediaminetetraacetic acid, pH 8.0, 0.5 mmol/L dithiothreitol, 50 mmol/L NaCl, 10 mmol/L Tris, pH 7.6, 50 μ g/ml poly (dl-dC); Pharmacia, Piscataway, NJ). For supershift analyses, antibodies to c-fos, c-jun, jun-B, and jun-D were added 15 minutes before adding the radiolabeled AP-1 oligonucleotide. Antibodies to c-jun, jun-B, and jun-D used in the EMSA experiments were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and antibodies to c-fos were obtained from Oncogene Research Products (Boston, MA). Reaction volumes were held constant to 15 μ l. Reaction products were separated in a 4% polyacrylamide gel and analyzed by autoradiography.

mRNA Expression Analysis

RNAs were extracted from lung homogenates using Trizol Reagent (Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer's protocol. Random primed reverse transcription for semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using Superscript II reverse transcriptase (Life Technologies, Inc.) following the manufacturer's instructions. Rat c-fos, c-jun, jun-B, jun-D, and housekeeping gene GAPDH mRNA expression was assessed using XpressPack mRNA expression analysis kits (Chemicon International, Temecula, CA) according to the manufacturer's protocols. Amplification reactions incorporating a biotinylated primer set generated the required amplification products. All primers are designed to span exon junctions and amplify only spliced RNA. Two μ l of the RT reaction was used for PCR amplification of c-fos, c-jun, jun-B, jun-D, and GAPDH using different annealing temperatures of 55°C, 58°C, 58°C, 58°C, and 60°C, respectively, as recommended in the protocols. Preliminary studies showed that 40-cycle amplification for c-fos and jun-B and 30-cycle amplification for c-jun and jun-D could

Figure 1. **A:** Time course (0 to 4 hours) for AP-1 activation during IgG immune complex-induced alveolitis. Nuclear extracts from whole lung tissues were subjected to EMSA analysis. **B:** Specificity of the AP-1 oligonucleotide probe. DNA-binding reactions with nuclear extracts from whole lungs harvested 4 hours after IgG immune complex deposition were used for competition assay with unlabeled (cold) AP-1 (50-fold excess) oligonucleotide and cold NF- κ B (50-fold excess) oligonucleotide. **C:** EMSA analysis of BAL macrophages for AP-1 in nuclear extracts harvested 0, 0.5, and 1.0 hour after IgG immune complex deposition. **D:** EMSA analysis of AP-1 activation in nonlavaged or lavaged lungs 4 hours after IgG immune complex deposition. Results shown in **A** to **D** are representative of three separate and independent experiments.

produce sufficient amplicon for detection without reaching saturated amplification (data not shown). GAPDH was used as a control for RNA integrity and RT efficiency in all experiments. PCR products were detected using Oligo-Detect colorimetric detection system (Chemicon International) following the manufacturer's instructions. Briefly, the single-stranded amplicon is captured to the plate via hybridization with a specific oligonucleotide probe immobilized to the reaction well. The hybrid complex is then reacted with 100 μ l of streptavidin conjugated to horseradish peroxidase. After washing, 100 μ l of 3,3',5,5'-tetramethylbenzidine (TMBE) substrate is added to each well for color development. Optical density was determined using a spectrophotometric plate reader at 450 nm. The intensity of color is directly proportional to the amount of amplicon present in samples.

Western Blot Analysis

Alveolar macrophages and whole lung extracts were obtained at the indicated time points relative to initiation of IgG immune complex deposition. Nuclear extracts were prepared as described above. Nuclear extracts were precleared with protein G beads coated with BSA overnight at 4°C with gentle rotation. Protein concentrations in nuclear extracts were determined by bicinchoninic acid assay with trichloroacetic acid precipitation using BSA as a reference standard (Pierce Co.). Samples containing 25 μ g of protein were electrophoresed in a denaturing 10% polyacrylamide gel and then transferred to a nitrocellulose membrane. Nonspecific binding sites were blocked with TBST (40 mmol/L Tris, pH 7.6, 300 mmol/L NaCl, 0.1% Tween 20) containing 5% nonfat dry milk for 12 hours at 4°C. Membranes were incubated with the

following antibodies in a 1:1000 dilution: polyclonal rabbit anti-rat c-fos (Ab-2) (Oncogene Research Products, Uniondale, NY), polyclonal rabbit anti-rat c-jun (H-79), polyclonal rabbit anti-rat jun-B (210), and polyclonal rabbit anti-rat jun-D (329) (Santa Cruz Biotechnology). After five washes in TBST, membranes were incubated in a 1:10,000 dilution of horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham). The membrane was developed by enhanced chemiluminescence technique according to the manufacturer's protocol (Amersham).

Statistical Analysis

Where indicated, data were analyzed using a one-way analysis of variance and individual group means were then compared with the Student-Newman-Keuls multiple comparison test. Differences were considered significant when $P < 0.05$.

Results

Activation of AP-1 during IgG Immune Complex-Induced Alveolitis

The kinetics of AP-1 activation during IgG immune complex-induced lung injury were evaluated by EMSA using nuclear extracts from whole lung obtained at various time points after the onset of lung injury. Barely detectable levels of AP-1 were observed in whole lung nuclear extracts at time 0 (Figure 1A). There was a modest increase after 2 hours and a clear increase at 4 hours. The specificity of the AP-1 consensus oligonucleotide probe was confirmed by experiments using nuclear extracts from

Figure 2. mRNA expression analysis of c-fos (A), c-jun (B), jun-B (C), and jun-D (D) in whole lung extracts after IgG immune complex deposition. RNA was extracted from lung homogenates of normal control (con) lungs and from lungs 0, 0.5, 1, 2, and 4 hours after initiation of reactions. mRNA expression of rat c-fos, c-jun, jun-B, jun-D, and GAPDH was assessed using XpressPack mRNA expression analysis kits, as described in Materials and Methods. Optical density was determined using a spectrophotometric plate reader at 450 nm. Band intensity was directly proportional to the amount of amplicon present in samples. GAPDH was used as a control for RNA integrity and RT efficiency in all experiments. Values represent mean $\pm$ SEM, with $n = 4$ per group.

whole lungs harvested 4 hours after IgG immune complex deposition (Figure 1B). Nuclear extracts incubated with 32 P-labeled AP-1 consensus oligonucleotide probe showed typical binding (left lane). AP-1 detection was totally lost in the presence of a 50-fold excess of an unlabeled oligonucleotide that binds to AP-1 (middle lane), whereas competition with excess (50-fold) of unlabeled NF- κ B oligonucleotide probe showed no reduction in AP-1 binding to DNA (right lane). When AP-1 activation was evaluated in BAL macrophages as a function of time, there was a clear increase in the intensity of AP-1 at 0.5 and 1.0 hour after onset of the lung inflammatory response (Figure 1C). Thus, the time frame for activation of AP-1 in macrophages and whole lung extracts is quite different.

To assess the contribution of BAL cells to AP-1 binding in whole lung homogenates, IC-injured lungs were lavaged several times, and AP-1 activation in nonlavaged or lavaged lungs was assessed. As shown in Figure 1D,

lavage of inflamed lung causes very limited reduction in AP-1 binding in whole lung homogenates. Densitometric analysis of AP-1 binding shows that there is a 15% reduction in AP-1 intensity in lungs after multiple lavages, but it did not reach a statistical significance, suggesting that BAL cells minimally contribute to AP-1 activation in whole lung nuclear extract.

Time Courses of c-fos, c-jun, jun-B, and jun-D mRNA Expression in Inflamed Lungs

Expression of mRNA for c-fos, c-jun, jun-B, and jun-D in lung homogenates was determined 0, 0.5, 1, 2, and 4 hours after intrapulmonary deposition of IgG immune complexes and was compared to mRNA extracted from normal lungs (con). The data are presented as optical densities (Figure 2). The left vertical axes represent optical density values for c-fos, c-jun, jun-B, and jun-D,

whereas the right vertical axes represent optical density values for GAPDH. Low constitutive levels of c-fos, c-jun, jun-B, and jun-D mRNA expression at time 0 were observed in lungs (optical densities ranging from 0.3 ~ 0.5) in comparison to negative controls (normal lungs) with optical densities of 0.2 ~ 0.3 ($P < 0.05$, $n = 4$). c-fos and jun-B mRNA expression peaked very early, at 0.5 hour, and remained at high levels through 2 hours, then dropping to basal levels by 4 hours (Figure 2, A and C). c-jun mRNA showed very transient expression, peaking at 0.5 hour and then decreasing rapidly to basal levels by 2 hours (Figure 2B). jun-D mRNA expression significantly increased at 0.5 hour, peaked at 1 hour, remained at high levels through 2 hours, then rapidly declined to basal levels after 4 hours (Figure 2D). In all cases, GAPDH mRNA values remained constant at all time points.

Expression of c-fos, c-jun, jun-B, and jun-D Proteins in Alveolar Macrophages and Whole Lungs

To identify the components of AP-1 in the inflamed lung and alveolar macrophages, we conducted a series of supershift assays on both whole lung nuclear extracts from IgG immune complex-injured lungs 4 hours after onset of injury and nuclear extracts from alveolar macrophages retrieved from inflamed lung 0.5 hours after IgG immune complex deposition. As shown in Figure 3A, the AP-1 complex in alveolar macrophages was supershifted by antibodies to c-jun, jun-B, or jun-D, whereas antibody to c-fos did not cause significant supershift. As shown in Figure 3B, the AP-1 complex in lung tissue was supershifted by antibodies to c-fos, jun-B, and jun-D, but not by c-jun antibody. These data suggest that there are different heterodimeric patterns of AP-1 in alveolar macrophages and lung tissues, and that c-jun is a major component in the formation of AP-1 complex in alveolar macrophages, but not in lung tissues.

To further analyze the formation of AP-1 complex, protein expression in nuclear extracts from alveolar macrophages and whole lungs was evaluated by Western blot analysis for c-fos, c-jun, jun-B, and jun-D. The results are shown in Figure 3C. In noninflamed lungs c-fos, jun-B, and jun-D were found neither in macrophage extracts nor in lung extracts. In noninflamed lungs, c-jun was constitutively expressed in nuclear extracts both from alveolar macrophages and whole lungs. In inflamed lungs, c-fos and jun-D protein levels were clearly increased, especially in extracts from whole lungs at 4 hours. c-jun protein was increased in nuclear extracts from BAL macrophages as well as lungs at 0.5 and 4 hours, respectively. jun-B protein was strongly expressed in extracts from both macrophages (at 0.5 hour) and lungs 4 hours after initiation of the inflammatory response. These data suggest that c-fos, c-jun, jun-B, and jun-D are robustly up-regulated components of AP-1, in this model of lung inflammatory injury.

Figure 3. **A:** Supershift analysis of AP-1 component in whole lung nuclear extract. DNA-binding reactions with nuclear extracts from whole lungs harvested 4 hours after IgG immune complex deposition were incubated with 32 P-labeled AP-1 oligonucleotide in the absence or presence of antibodies to the AP-1 proteins (c-fos, c-jun, jun-B, jun-D). **B:** Supershift analysis of AP-1 component in alveolar macrophages. DNA-binding reactions with nuclear extracts from alveolar macrophages harvested 0.5 hour after IgG immune complex deposition were incubated with 32 P-labeled AP-1 oligonucleotide in the absence or presence of antibodies to the AP-1 proteins (c-fos, c-jun, jun-B, jun-D). **C:** Western blot analysis of c-fos, c-jun, jun-B, and jun-D protein in nuclear extracts from alveolar macrophages (at 0 and 1 hour) and in lung nuclear extracts (at 0 and 4 hours) after onset of inflammation. The nuclear extracts (containing 25 μ g of protein) were electrophoresed in a denaturing polyacrylamide gel and then transferred to a nitrocellulose membrane. Protein expression by Western blot analysis was evaluated by using the following antibodies: polyclonal rabbit anti-rat c-fos, polyclonal rabbit anti-rat c-jun, anti-rat jun-B, and polyclonal goat anti-rat jun-D. The graphs are representative of findings in two separate and independent experiments.

Effects of Alveolar Macrophage Depletion on Lung AP-1 Activation

Because these studies suggest that AP-1 activation in alveolar macrophages is a very early event in IgG immune complex-induced lung injury, we sought to determine whether alveolar macrophage depletion could affect AP-1 activation in whole lung tissues. Nuclear extracts of whole lungs harvested 4 hours after IgG immune complex deposition were analyzed by EMSA. Lungs from rats pretreated with PBS-liposome or Cl_2MDP -liposomes and subsequently challenged intratracheally with PBS showed little if any evidence of AP-1 activation (Figure 4, PBS lungs, lanes 1 to 4). In rats pretreated with PBS-liposomes and challenged with IgG immune complexes, (IgG-IC), lung AP-1 activation dramatically increased (lanes 5 and 6). Depletion of alveolar macrophages with Cl_2MDP -liposomes markedly reduced the extent of lung AP-1 activation induced by IgG immune

Figure 4. Effects of lung macrophage depletion on AP-1 activation in whole lung nuclear extracts 4 hours after intratracheal challenge with PBS (lanes 1 to 4) or immune complexes (lanes 5 to 8). Twenty-four hours earlier, animals received an intratracheal instillation of PBS-liposomes or Cl_2MDP -liposomes and AP-1 activation was determined by EMSA. Results are from duplicate animals and are representative of data from two separate and independent experiments.

complexes (lanes 7 and 8). Thus, lung macrophages seem to be vital for immune complex-induced up-regulation of AP-1.

Requirement for TNF- α , But Not Complement, in Lung AP-1 Activation Induced by Pulmonary Deposition of IgG Immune Complexes

Because depletion of alveolar macrophages suppressed AP-1 activation and it is well known that macrophage-derived TNF- α is an important cytokine that promotes acute lung injury, we examined the requirement TNF- α in AP-1 activation. Neutralizing antibodies to TNF- α were administered intratracheally with the anti-BSA and AP-1 activation was assessed in whole lung nuclear extracts or alveolar macrophages by EMSA 4 hours or 0.5 hour after initiation of lung inflammation, respectively. The administration of neutralizing antibodies to TNF- α clearly attenuated lung activation of AP-1 (Figure 5A). However, blockade of TNF- α had no effects on AP-1 activation in alveolar macrophages retrieved from lungs 0.5 hour after IgG immune complex deposition (data not shown).

The activation of complement and subsequent generation of C5a has been shown to be critical in this model for the development of the inflammatory response,¹⁵ and the expression of multiple cytokines and chemokines, which are under the control of AP-1, has been shown to be enhanced by complement activation.¹⁶ To examine

Figure 5. Effects of TNF- α blockade (A) and complement depletion (B) on AP-1 activation after intrapulmonary deposition of IgG immune complexes. For TNF- α blockade, 300 μg of anti-TNF- α IgG or control IgG were administered intratracheally with the anti-BSA. Complement depletion in rats was accomplished by intraperitoneal injection of 25 U of purified cobra venom factor at 36, 24, and 12 hours before the induction of IgG immune complex-induced lung injury. For both experiments, AP-1 activation was evaluated by EMSA of whole lung nuclear extracts harvested 4 hours after initiation of the reactions. Results shown are from duplicate experiments.

whether complement activation was required for pulmonary activation of AP-1, rats were depleted of complement by serial intraperitoneal injections of purified cobra venom factor. The systemic depletion of complement did not reduce lung AP-1 activation induced by IgG immune complex deposition (Figure 5B), similar to what has been found for NF- κB activation in the same lung inflammatory model.⁹ These data indicate that endogenous TNF- α induced by IgG immune complex deposition, but not complement activation products, contributes to AP-1 activation during IgG immune complex-induced lung injury.

Discussion

In this study, we have demonstrated that AP-1 activation in lung is associated with induction of the acute lung inflammatory response induced by intrapulmonary deposition of IgG immune complexes. Depending on its composition, the AP-1 complex seems to be associated with functional differences in cell growth, cell activation, and apoptosis.¹⁷ In the current report, we show that c-fos, c-jun, jun-B, and jun-D were protein components of the AP-1 complex appearing in nuclear extracts from IgG immune complex-injured lung and BAL macrophages. Western blot analysis revealed that c-jun was more strongly expressed in alveolar macrophages, as compared to whole lung extracts. This conformational disparity may imply different functions associated with AP-1 activation in alveolar macrophages and other lung cell types during IgG immune complex-induced lung injury. Additionally, our results indicate that c-fos and jun-B may be the predominant proteins of the AP-1 complex in nuclear extracts from inflamed rat lungs. jun-B may play a regulatory role in the AP-1 downstream signaling in inflamed lungs, given that jun-B can be either a repressor or an activator of transactivation that is regulated by AP-1.^{18–21} c-jun mRNA expression showed more tran-

sient up-regulation than that for c-fos, jun-B, and jun-D during IgG immune complex-induced injury. Further study is needed to precisely define the roles of AP-1 components in lungs and alveolar macrophages during IgG immune complex-induced injury.

Alveolar macrophages play an important role in host defense and in processes of inflammation in the lung. Central to the lung inflammatory response is the production of TNF- α , IL-1, and other cytokines and chemokines by alveolar macrophages.⁸ Our current data demonstrate that pulmonary AP-1 activation occurs initially in alveolar macrophages, suggesting that products of activated alveolar macrophages may propagate the inflammatory response in other lung cell types through activation of AP-1. Depletion of alveolar macrophages prevented IgG immune complex-induced AP-1 activation in lung tissues. We have previously reported in the same model that macrophage depletion greatly suppresses NF- κ B translocation induced by IgG immune complexes.¹³ Others have shown that gliotoxin, the diffusible product from *Aspergillus fumigatus*, inhibits the production of proinflammatory cytokine expression in alveolar macrophages by suppressing activation of both AP-1 and NF- κ B.²² Deletion and point mutation analyses of the IL-8 promoter revealed that IL-8 induction by hypoxia required the cooperation of NF- κ B and AP-1.²³ NF- κ B and AP-1 cooperate to mediate IL-1-induced collagenase-1 (MMP-1) gene transcription.²⁴ Thus, it is likely that in the inflamed lung cytokine gene expression requires participation of both AP-1 and NF- κ B. What remains to be determined is the extent to which AP-1 and NF- κ B activation is linked.

TNF- α induces the activation of NF- κ B and AP-1 in alveolar epithelial cells.²⁵ TNF- α promoter itself contains NF- κ B and AP-1 binding sites and is subject to positive autoregulation.²⁶ These data suggest the presence of a positive feedback loop by which TNF- α can amplify the lung inflammatory response through activation of both AP-1 and NF- κ B. The current data indicate that blockade of endogenous TNF- α with antibody prevents IgG immune complex-induced AP-1 activation in lung, but had no effects on AP-1 activation in alveolar macrophages 0.5 hour after IgG immune complex deposition. These data suggest that AP-1 activation in lung tissues is TNF- α -dependent, and alveolar macrophages may use a TNF- α -independent pathway to activate AP-1. The development of IgG immune complex-induced lung injury is known to be dependent on complement activation products. Our previous studies have shown that complement activation products are required for full production of TNF- α and chemokines.^{15,16} However, we now demonstrate that complement depletion has no effect on pulmonary activation of AP-1, which is consistent with our recent findings that complement is also not required for activation of NF- κ B in lung.^{9,16} It is clear that TNF- α promotes lung inflammation through the activation of both AP-1 and NF- κ B, whereas complement activation products do so by a mechanism that is independent of either AP-1 or NF- κ B.

The role of AP-1 in the development of acute lung inflammatory injury induced by IgG immune complexes seems to be one featuring collaboration with NF- κ B. c-

fos, c-jun, and jun-D can directly interact with the p65 subunit of NF- κ B.^{27,28} Both of these transcription factors regulate a large number of proinflammatory mediators, and, in fact, the transcription of many of these mediators requires cooperative transactivation of AP-1 and NF- κ B for optimal gene expression.^{9,16,27,29,30} In our model, AP-1 and NF- κ B share an almost superimposable time course of activation (Figure 1).⁹ Similarly, both AP-1 and NF- κ B are activated very early (by 30 minutes) in alveolar macrophages after deposition of IgG immune complexes and thus seem to be required for the initiation of this lung inflammatory response. Thus, it is likely that AP-1 cooperates with NF- κ B to orchestrate inflammatory responses by regulating gene expression. This hypothesis is currently under investigation.

In summary, the present studies have implicated the transcription factor, AP-1, in inflammatory events in lung after deposition of IgG immune complexes. It seems that AP-1, together with NF- κ B, is responsible for the production of inflammatory mediators and the subsequent dissemination of the inflammatory response from alveolar macrophages to other cells within the lung. A further understanding of the relationship between AP-1 and NF- κ B may reveal critical clues to the pathogenesis of acute lung injury and identify potential sites of therapeutic intervention.

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