A role for C-C chemokines in fibrotic lung disease

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Abstract: Pulmonary fibrosis is the end point of a chronic inflammatory process characterized by leukocyte recruitment and activation, fibroblast proliferation, and increased extracellular matrix production. Previous studies of models of pulmonary fibrosis have investigated the role of cytokines in the evolution of the fibrotic response. The involvement of tumor necrosis factor and interleukin-1 in bleomycin-induced lung injury, a model of idiopathic pulmonary fibrosis, has been well established, suggesting that cytokines mediate the initiation and maintenance of chronic inflammatory lesions. However, the aforementioned cytokines alone cannot account for the recruitment and activation of specific leukocyte populations found in the bleomycin model. Recently, a family of novel proinflammatory cytokines (chemokines) was cloned and characterized, yielding many putative mediators of leukocyte functions. Macrophage inflammatory protein-1α (MIP-1α) and monocyte chemoattractant protein-1 (MCP-1) belong to the C-C chemotactic cytokine family, a group of low-molecular-weight peptides. These molecules modulate chemotaxis, proliferation, and cytokine expression in leukocyte subsets. Our group has investigated the roles of MCP-1 and MIP-1α in the bleomycin model. Both MCP-1 and MIP-1α are expressed in a time-dependent manner after bleomycin challenge, and passive immunization of these animals with either anti-MIP-1α or anti-MCP-1 antibodies attenuated leukocyte accumulation. In addition, we have identified specific cell types expressing MCP-1 or MIP-1α by in situ hybridization and immunohistochemical localization, respectively. Furthermore, our results indicate that MIP-1α expression is mediated by alveolar macrophage-derived tumor necrosis factor, identifying an important cytokine pathway in the initiation of pulmonary fibrosis. Finally, anti-MIP-1α therapy attenuated fibrosis, providing direct evidence for its involvement in fibrotic pathology. Our work has clearly established that the C-C chemokines MCP-1 and MIP-1α are expressed and contribute to the initiation and maintenance of the bleomycin-induced pulmonary lesion.*J. Leukoc. Biol.* 57: 782–787; 1995.

Key Words: *bleomycin • MCP-1 • MIP-1α • fibrosis • TNF • IPF • inflammation*

INTRODUCTION

Bleomycin sulfate, a drug used for the treatment of various malignancies, is a group of copper-containing, sulfated glycopeptides possessing significant antibiological activity. A side effect of bleomycin administration is acute pneumonitis, with a small fraction of these patients progressing to chronic pulmonary fibrosis, a condition resembling idiopathic pulmonary fibrosis (IPF) [1, 2]. In rodents, bleomycin administration results in a route-, dose-, and strain-dependent pulmonary inflammatory response [3–6]. Given intratracheally, this response is characterized by increases in leukocyte accumulation, fibroblast proliferation, and collagen content [7, 8]. Rodents challenged with bleomycin typically develop acute alveolitis 2–3 days postchallenge, followed by intense interstitial inflammation at 4–12 days [9–12]. Fibroblast proliferation and extracellular matrix synthesis is initiated 4–14 days postbleomycin challenge and collagen content is elevated approximately twofold, 3 weeks postchallenge [4–6, 10, 12]. Beyond 3 weeks postchallenge, fibrosis progresses, compromising respiratory function, resulting in significant morbidity in the animal. The rodent pulmonary inflammatory response to intratracheal bleomycin challenge constitutes a representative model of human IPF.

Since the initial characterization of the rodent model in 1974, many soluble mediators have been implicated in the pulmonary response to intratracheal bleomycin challenge [7, 13]. Bleomycin has been shown to induce free radical formation, resulting in lung lipid peroxidation and injury [14, 15]. Other putative soluble mediators are arachidonic acid metabolites. Inhibition of cyclooxygenase with indomethacin or inhibition of lipoxygenase with nordihydroguiaretic acid attenuated the fibrotic lesion, suggesting a role for the products of these pathways [16, 17]. Subsequent studies identified fibroblast growth-promoting factors from the lavage fluid and alveolar macrophage culture supernatants of bleomycin-challenged animals. One of these factors, originally characterized as macrophage-derived growth factor, is probably a combination of tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and other growth factors [13]. Various in vitro studies have identified fibroblast growth factor, TNF-α, and PDGF as potent inducers of fibroblast proliferation [18]. In contrast, other in vitro work has identified TGF-β as both an inhibitor and promoter of fibroblast proliferation, this disparate effect being dependent on the concentration of the cytokine and the source of the target cells [13, 18–20]. Further-

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Abbreviations: IPF, idiopathic pulmonary fibrosis; TNF, tumor necrosis factor; IL-1, interleukin-1; TGF-β, transforming growth factor-β; PDGF, platelet-derived growth factor; MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein.

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Received November 18, 1994; accepted January 12, 1995.

more, PDGF and TGF-β have been shown to induce collagen mRNA, whereas IL-1 is believed to increase collagen content via inhibition of collagenase activity [13].

In vivo time-dependent expression of TNF and IL-1 mRNA has been detected in whole lung and bronchoaveolar lavage preparations [12, 21, 22]. Initial expression of these mediators 3–8 days postbleomycin challenge precedes proliferation of fibroblasts and induction of collagen synthesis, which peak in the 2nd week. Interestingly, neutralization of TNF with anti-TNF antibodies or soluble TNF receptors abrogates the pulmonary fibrotic response in mice [12, 23]. Similarly, continuous intravenous infusion of bleomycin-challenged mice with IL-1 receptor antagonist protein resulted in a moderate reduction of fibrosis [24]. These results suggest that mediators such as TNF and IL-1, present in the 1st week postchallenge, have a role in the initiation of the lesion. Attenuation of the bleomycin-induced lesion after the inhibition of these distinct inflammatory mechanisms indicates that this response involves many different interdependent pathways by using a diverse milieu of soluble mediators.

The inflammatory response to bleomycin challenge may be described temporally by the appearance of different leukocyte subsets within the lungs. In mice and rats neutrophil accumulation peaks around 2 days postchallenge [9, 10]. Eosinophilic lung infiltrates have been observed 7 days postchallenge in both rats and hamsters [9, 11, 16, 25]. Bleomycin-challenged, mast cell-deficient, or neutrophil-depleted mice developed pulmonary fibrosis, despite these cellular deficiencies [26, 27]. In contrast to granulocytes, significant increases in mononuclear phagocytes are not observed until 4 days postchallenge, peaking at 8–14 days, depending on the dose of bleomycin and the strain of animal [9, 10]. Lung macrophages have been shown to produce arachidonic acid metabolites and reactive oxygen species, as well as TNF-α and IL-1 protein, on direct stimulation with bleomycin, underlying the importance of these cells to the development of the lesion [28, 29]. Elevated lymphocyte counts have been observed in lavage fluid as early as 2 days postchallenge and after 8 days the total lymphocyte count in the lungs approximates untreated controls [10, 25, 30]. Furthermore, bleomycin-challenged nude mice have diminished fibrosis compared with positive controls, and more recent CD4 and CD8 T lymphocyte depletion studies demonstrated abrogation of pulmonary fibrosis [12, 31]. In summary, these results suggest a central role for macrophages and lymphocytes, and little, if any, for neutrophils and mast cells, in the development of bleomycin-induced pulmonary pathology.

C-C chemokines: novel proinflammatory polypeptides

Recruitment of leukocytes is an essential step in the development of any inflammatory lesion. In small venules leukocytes bind endothelium via the expression of adhesion molecules on both leukocytes and endothelial cells. Adherent leukocytes extravasate out of the vascular compartment and migrate through the basement membrane and extracellular matrices to the site of inflammation. Once at the inflammatory lesion, recruited leukocytes modulate the host response in a stimulus-specific manner by cell-to-cell interactions and secretion of soluble mediators. The recruitment of leukocytes by the aforementioned processes is currently an area of intense scrutiny. Recently, a family of novel proinflammatory cytokines has been cloned and characterized, yielding many putative mediators of macrophage-, lymphocyte-, and granulocyte-derived responses in disease models [32, 33]. The C-C chemokine supergene family includes macrophage inflammatory protein-1α (MIP-1α), MIP-1β, RANTES, monocyte chemoattractant protein-1 (MCP-1), MCP-2, MCP-3, HC-14, C10, and I-309. C-C chemokines, grouped on chromosome 17, are so named because of the juxtaposition of the first two cysteine residues at the NH_2-terminal. Originally described as chemotactic factors, C-C chemokines also modulate cytokine production, adhesion molecule expression, and mononuclear cell proliferation [34–37]. Although mϕ and lymphocytes are the predominate cell types targeted, members of this family possess differential chemotactic and stimulatory activity for specific leukocyte subsets.

MIP-1α and MCP-1

MIP-1 was originally described as a 16-kDa lipopolysaccharide-inducible heparin-binding dipeptide (MIP-1α and -1β) with leukocyte chemotactic activity [38]. In vitro studies have shown that MIP-1α is chemokinetic for neutrophils and chemotactic for mononuclear phagocytes, basophils, and subsets of lymphocytes [38–40]. MIP-1α preferentially mediates chemotaxis of $CD8^+$ lymphocytes compared with $CD4^+$ lymphocytes, whereas the reverse is true for MIP-1β [40]. Appropriately stimulated T lymphocytes, alveolar and peritoneal macrophages, neutrophils, monocytes, airway epithelial cells, and fibroblasts have been identified as cellular sources of MIP-1α protein [10, 41–46]. MIP-1α stimulates IL-1, IL-6, and TNF production from peritoneal macrophages and MIP-1α protein expression from mononuclear cells is stimulated in vitro by lipopolysaccharide and IL-1 [34, 43, 46].

Initially characterized as the JE gene, MCP-1 is a glycosylated, 14- to 25-kDa protein cloned from PDGF-stimulated murine 3T3 cells [47]. Subsequent in vitro studies demonstrated stimulus-specific induction of MCP-1 mRNA from human fibroblasts and endothelial cells [48]. Originally described for its monocyte chemotactic activity, MCP-1 is a more potent chemoattractant for lymphocytes in vitro [49]. In addition, MCP-1 possesses chemotactic activity for basophils but not for neutrophils [39]. Peritoneal macrophages, lymphocytes, smooth muscle cells, epithelial cells, endothelial cells, and various transformed cell lines have been reported to secrete MCP-1 protein in the presence of serum or specific stimuli [32, 50]. MCP-1 stimulates the expression of IL-6 and IL-1 from peripheral blood mononuclear cells but has no effect on TNF expression [35].

In vivo MCP-1 and MIP-1α contribute to acute and cellular immune tissue responses via recruitment and activation of mononuclear phagocytes and T lymphocytes. Elevated MIP-1α has been measured as early as 6 h postlipopolysaccharide challenge in the lungs of CD-1 mice and elevated MCP-1 expression has been reported at early time points in a model of dermal wound repair [36, 51]. Passive immunization of granuloma-bearing CBA/J mice with either anti-MIP-1α or anti-MCP-1 antibodies decreased the size and cellularity of the inflammatory granulomatous lesion [52, 53]. These results provide evidence that increases in MCP-1 and MIP-1α expression contribute to the development of both acute and chronic inflammatory lesions.

C-C chemokines in fibrotic lung disease

The clinical diagnosis of IPF is based on the presence of dyspnea, a radiographic profusion score, and pulmonary function tests. Consequently, because diagnosis is made after the onset of findings caused by fibrotic pathologies, studies of the initiation of the fibrotic lesion have been difficult in humans. Nevertheless, analysis of bronchoalveolar lavage and open lung biopsies from patients who meet the diagnostic criteria for IPF has indicated elevated levels of MCP-1 and MIP-1α protein compared with healthy volunteers. In addition, anti-MCP-1 and anti-MIP-1α antibodies inhibited 22% and 18%, respectively, of the mononuclear cell chemotactic activity in lavage fluid [41]. Fibroblast cultures grown out of fibrotic regions obtained by open lung biopsies from IPF patients constitutively secreted significant levels of MCP-1 and MIP-1α protein. In contrast, normal fibroblast cultures derived from the tissue of normal regions of lungs did not express chemokines in a similar manner [41]. This result indicates that the initial inflammatory processes in IPF may promote formation of an altered phenotype of lung fibroblasts.

C-C chemokine expression in the bleomycin model

Rodent models have previously been evaluated at later time points (3 weeks) postbleomycin challenge, providing information on the maintenance stage of the fibrotic lesion. However, recent rodent studies have focused on the involvement of C-C chemokines in the early stage (3 weeks) after bleomycin administration, providing information on the initiation of the fibrotic lesion [54]. Time-dependent expression of MCP-1 mRNA has been reported in response to bleomycin challenge in rats (**Table 1**). MCP-1 mRNA extracted from alveolar lavage cells peaked at 24 h postchallenge, whereas the signal from whole lung homogenates peaked at 7 days, correlating with eosinophil, lymphocyte, and mononuclear phagocytes accumulation [25]. Interestingly, similar to MCP-1, TNF and IL-1 protein are differentially expressed by alveolar and interstitial macrophages isolated from bleomycin-challenged mice, providing a putative cellular basis for the compartment-specific expression of MCP-1 protein [21].

In CBA/J mice we have observed elevated MCP-1 protein 1, 2, and 4 days postchallenge in whole lung homogenates, with little or no detectable increases at 8–21 days (Table 1). In addition, we have measured elevated MCP-1 protein 2 days postchallenge in bronchoalveolar lavage Fluid (BALF). Similar to the compartment-specific expression in the rat, MCP-1 is elevated in whole lung homogenates but not in lavage fluid 4 days postbleomycin challenge (unpublished observations). MIP-1α protein and mRNA (**Fig. 1**) expression in lung homogenate preparations was elevated at 1, 2, 8, 12, and 16 days postchallenge, with detectable antigen peaking at 2 and 16 days (Table 1) [10]. In contrast to MCP-1, the kinetics of whole lung MIP-1α expression approximated the expression in BALF. In the murine model the kinetics of expression of MIP-1α and MCP-1 during the 1st week postchallenge coincide temporally with the accumulation and trafficking of lymphocytes and mononuclear phagocytes, which are first elevated at 2 and 4 days, respectively. In the 2nd week postchallenge, MIP-1α expression coincides with large increases in mononuclear phagocytes, whereas elevated MCP-1 expression is not observed at this time. These data and the previous findings in the rat provide circumstantial evidence for MCP-1 and MIP-1α acting as chemotactic factors in vivo.

Although the above data are intriguing, they do not provide direct evidence for the actual functions of MCP-1 and MIP-1α in vivo. Neutralization of these chemokines during the response to bleomycin has yielded interesting results. Passive immunization of CBA/J mice with anti-MCP-1 (unpublished observations) or anti-MIP-1α antibodies reduced total lung inflammatory cell content by 30% and 35%, respectively [10]. Differential analysis indicated reduction of mononuclear cells by anti-MCP-1, whereas both mononuclear phagocytes and, to a lesser extent, granulocytes were decreased after anti-MIP-1α treatment. Consistent with these results, anti-MIP-1α reduced the total number of Mac-1 (CD11b)-positive staining cells compared with positive controls (**Table 2**). These results are consistent with the reported in vitro chemotactic activities of both MCP-1 and MIP-1α and with the specific chemotactic activities attributed to these chemokines in the lavage fluid of IPF patients.

Roles for mononuclear phagocytes and lymphocytes in the bleomycin model have been clearly established. Less well characterized, however, is the role of B cells. Immune complex deposition contributes significantly to the pathological processes observed in IPF but has not been well evaluated in the rodent model. In bleomycin-challenged mice we have detected increases in cells labeling positive for the B220 antigen, a marker for B cells (Table 2). Interestingly, passive immunization of challenged mice with anti-MIP-1α antibodies decreased the fraction of lymphocytes staining positive for B220 from 42% to 36%

TABLE 1. Summary of Cytokine Expression and Function in Bleomycin-Induced Lung Injury

Expression/Function	Animal	TNF	IL-1	MIP-1α	MCP-1
Expressed early (week 1)	Mouse	+	+	+ + +	+ +
	Rat	+	+	ND	+ +
Expressed late (week 2)	Mouse	+ +	+ +	+ + +	—
	Rat	+ +	+ +	ND	+ +
Effects of passive immunotherapy on					
Inflammation	Mouse	+ + +	ND	+ +	+ +
Fibrosis	Mouse	+ + + +	+ +	+ +	ND

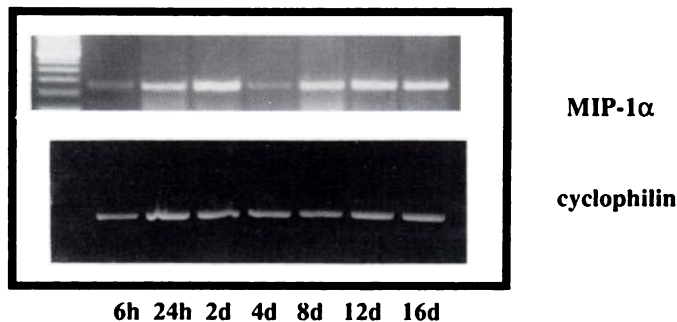

Fig. 1. Detection of elevated MIP-1α mRNA by reverse transcription-polymerase chain reaction in whole lung homogenates from CBA/J mice 6 h to 16 days postbleomycin challenge.

TABLE 2. Flow Cytometric Analysis of Whole Lung Homogenates from Bleomycin, Preimmune Serum or Anti-MIP-1α Immune Serum-Treated CBA/J Mice

Intraperitoneal treatment	Flow cytometry marker				
	CD3	CD4	CD8	B220	Mac-1
Preimmune serum	8.2 ± 1.0	6.7 ± 0.6	1.2 ± 0.2	3.3 ± 2.0	4.9 ± 1.0
Anti-MIP-1α polysera	8.0 ± 1.2	5.6 ± 0.9	0.9 ± 0.1	2.2 ± 1.1	3.2 ± 0.7

Date expressed as millions of net inflammatory cells ([total inflammatory cells × percentage of each leukocyte marker] − treatment matched negative control), n = 3.

(data not shown), corresponding to a net decrease of approximately one million cells per animal (Table 2). These results suggest that MIP-1α mediates B cell accumulation during bleomycin-induced lung injury.

Chemotaxis may not be the only proinflammatory mechanism attributed to MIP-1α and MCP-1 in vivo. Recent work suggests MIP-1α modulates intercellular adhesion molecule-1 expression, whereas MCP-1 can stimulate dose-dependent expression of CD11b and CD11c on the surface of peripheral blood monocytes, suggesting that chemokines promote adhesion events in vivo [35, 36]. In addition, peritoneal macrophages proliferated in vitro when exposed to MIP-1α alone or to MIP-1α with colony-stimulating factor-1 or granulocyte-macrophage colony-stimulating factor [37]. Although the precise mechanisms are unknown, the above results indicate that MCP-1 and MIP-1α contribute to the processes of leukocyte accumulation in the bleomycin-induced pulmonary fibrosis model.

Identification of cellular sources of C-C chemokines in vivo

Many in vitro studies have identified cellular sources of C-C chemokines. More important, however, is determining which cell type(s) are sources in vivo. Alveolar macrophages from bleomycin-challenged mice have been identified as a possible cellular source of MCP-1 by immunohistochemical localization (unpublished observations). Also, in the murine model, alveolar macrophages, airway epithelial cells, and interstitial macrophages stained positive for MIP-1α antigen [10]. Alveolar macrophages were positive for MIP-1α as early as 24 h postchallenge, whereas interstitial macrophages were not positive until day 8, correlating with the progression of the disease process from the alveolar to the interstitial space. Recently, eosinophils were identified as a potential source of MCP-1 by in situ hybridization [25]. In the rat lung eosinophils accounted for 85% of MCP-1-positive cells 7 days postbleomycin challenge. Peaking at day 7, MCP-1-positive eosinophils persist throughout the proliferative phase (2nd week) of the lesion. This result establishes an important cellular source of MCP-1 and argues that eosinophils, unlike neutrophils and mast cells, potentially have a role in the evolution of the fibrotic response.

The identification of eosinophil participation in the rat bleomycin lesion provides an in vivo basis for recent receptor studies performed on the human eosinophilic HL-60 cell line. Butyric acid induced expression of a RANTES and MIP-1α dual receptor on differentiated HL-60s, and this receptor mediated chemotactic Ca^{2+} flux and respiratory burst responses [55]. Complementing this result, our lab has recently demonstrated that MIP-1α mediates eosinophil accumulation in a murine model of airway hyperactivity [44]. Although complete investigations have yet to be conducted in the same model, these data provide a mechanism for eosinophil recruitment and participation in chronic inflammatory processes.

Fibroblast-derived C-C chemokines in the bleomycin-induced lesion

The 2nd week of postbleomycin challenge is the period in which increases in fibroblast proliferation and collagen mRNA occur. Recently, cells with a myofibroblast phenotype, as determined by expression of α-smooth muscle actin, were shown to be the predominate cell type responsible for procollagen mRNA expression 7–14 days postchallenge [56]. In related experiments, α-smooth muscle actin-positive cells were also positive for MCP-1 mRNA [25]. These results identify a potential target for profibrotic stimuli within the bleomycin lesion. Consistent with this conclusion, our lab has isolated C-C chemokine-secreting, fibroblast-like cells from schistosoma egg-induced liver granulomas [43]. These cells constitutively expressed MCP-1 mRNA and protein, whereas MIP-1α mRNA and protein was expressed in a stimulus-specific manner. These results indicate that fibroblasts from rodent models of chronic inflammation appear to have an altered phenotype, similar to fibroblast explants from IPF patients.

Fibroblast-derived MCP-1 or MIP-1α may have several different functions in the fibrotic lesion. Although there is no evidence indicating that MCP-1 or MIP-1α directly induce fibroblast proliferation or collagen synthesis, they could be competence factors for these activities in vivo. In addition, fibroblast-derived C-C chemokines may contribute to the maintenance of the fibrotic lesion by initiating mononuclear cell accumulation and by modulating expression of profibrotic mediators from the surrounding granulation tissue. To evaluate these putative mechanisms, we passively immunized CBA/J mice with anti-MIP-1α antibodies and measured hydroxyproline content 21 days postbleomycin challenge. Bleomycin-challenged mice treated with anti-MIP-1α antibodies demonstrated a 49% decrease in lung hydroxyproline compared with bleomycin-challenged mice treated with preimmune serum [10]. These results support the notion that C-C chemokines participate in the fibrotic process, possibly by inducing leukocyte accumulation and activation.

Cytokine networks mediate the response to bleomycin

Several studies have illustrated a central role for TNF in the murine bleomycin model. Nevertheless, linkage of TNF expression and specific leukocyte recruitment has not been previously demonstrated. We were able to detect bioactive TNF but not MIP-1α in lavage fluid of mice 6 h after bleomycin challenge (unpublished observations). Furthermore, elevated TNF protein and mRNA has been previously detected 3–8 days postchallenge, establishing that TNF is increased before both the early (2 day) and late (16 day) MIP-1α peaks [12, 21]. Supporting these results, normal lavage cells from CBA/J mice express MIP-1α protein after stimulation with TNF in vitro. Recent experiments in our laboratories suggest that TNF is a stimulus for MIP-1α expression in vivo. In CBA/J mice treated with soluble TNF receptor we detected an 80% reduction of MIP-1α protein 2 days postbleomycin chal-

lenge, a time corresponding to the first MIP-1α expression peak (unpublished observations). In addition, mice treated with soluble TNF receptor at 4 days postbleomycin administration (after the first MIP-1α peak) demonstrated a modest 35% reduction in MIP-1α at day 10 postchallenge, a time point corresponding to the second MIP-1α expression peak. However, previous data have established that IL-1 is a potent stimulus for MIP-1α in vitro [43, 46]. Because TNF can induce IL-1, TNF may stimulate MIP-1α expression indirectly. Studies are underway in our lab to evaluate the potential costimulatory role of IL-1 in TNF-mediated MIP-1α production during the pulmonary response to bleomycin challenge.

Recent studies in the chronic schistosoma granuloma model support the notion of a TNF-mediated MIP-1α pathway. Similar to the bleomycin pulmonary lesion, primary liver schistosoma granuloma formation may be attenuated with either anti-MIP-1α or anti-TNF antibody therapy [52]. Interestingly, IL-1 receptor antagonist protein is also elevated in the primary granuloma lesion, suggesting a lesser role for IL-1 and a greater role for TNF in the initiation of the chronic granulomatous response [57]. In summary, the cytokine expression patterns observed in the primary schistosoma granuloma, along with data from the bleomycin model, support the contention of a TNF-mediated MIP-1α pathway for the initiation of chronic inflammatory lesions.

Chronic inflammatory processes, like the bleomycin-induced lesion, are postulated to involve a macrophage-lymphocyte-fibroblast cellular axis responsible for the initiation and maintenance of chronic inflammation. In the bleomycin model inhibition of lymphocyte-, macrophage-, or fibroblast-mediated inflammatory mechanisms attenuates fibrosis, the hallmark of the lesion. It may be postulated that these cell types activate one another, initiating and maintaining the chronic lesion via secretion of soluble proinflammatory substances. The data discussed in this review indicate that macrophages, lymphocytes, and fibroblasts both produce and are modulated by MCP-1 and MIP-1α, suggesting a role for C-C chemokines in this putative cellular network. In this context, future experiments will examine the specific roles of MCP-1, MIP-1α, and additional chemokines in lung fibrosis, leukocyte recruitment, and maintenance of the chronic bleomycin-induced lesion. Regardless of the specifics of this cellular network, C-C chemokines are expressed and regulate chronic lung injury during the response to bleomycin challenge.

ACKNOWLEDGMENTS

This research was supported in part by National Institutes of Health grants HL02401, HL31693, HL28737, HL52285, 1P50HL46487, HL35276, and HL50057.

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