Macrophage/fibroblast coculture induces macrophage inflammatory protein-1α production mediated by intercellular adhesion molecule-1 and oxygen radicals

Matthew L. Steinhauser,* Steven L. Kunkel,* Cory M. Hogaboam,* Holly Evanoff,* Robert M. Strieter,† and Nicholas W. Lukacs*

*University of Michigan Medical School, Departments of *Pathology and †Internal Medicine, Division of Pulmonary and Critical Care, Ann Arbor, Michigan*

Abstract: This study examined the cell-to-cell interaction between fibroblasts and macrophages as a possible contributor to the chronic inflammatory state. In a coculture system, consisting of macrophages layered over confluent fibroblasts, there was a significant increase in macrophage inflammatory protein 1α (MIP-1α) compared with control cultures. ICAM-1 adhesion was identified as an important stimulus of MIP-1α production by using ICAM-1-specific monoclonal antibodies. Furthermore, fibroblasts from ICAM-1 knockout mice induced significantly less MIP-1α production from peritoneal macrophages when compared to control fibroblasts. In addition, it appeared that oxygen radicals functioned as activating molecules during cellular interaction and production of MIP-1α, as the addition of the antioxidant *N*-acetylcysteine (NAC) prevented MIP-1α secretion. Thus, the ICAM-1 and oxygen radical-mediated induction of MIP-1α associated with a macrophage/fibroblast coculture system provides one possible mechanism by which immune/inflammatory cell interactions may augment chemokine production and exacerbate chronic inflammatory diseases. *J. Leukoc. Biol.* 64: 636–641; 1998.

Key Words: *chemokines · monoclonal antibodies*

INTRODUCTION

Inflammatory/immune cell influx is a necessary response to pathogenic invaders, facilitating the timely eradication of pathogens. Often, however, after the infecting agent has been cleared, the immune response persists and immune cells continue to infiltrate previously infected tissues. This pattern of chronic inflammation has been extensively documented, especially in the context of fibrotic diseases [1]. Furthermore, the persistent recruitment of monocytes to fibrotic tissue has been identified as a major component in the progression of chronic inflammatory diseases [2]. Leukocyte recruitment is initiated by adhesion molecules on the surfaces of endothelial cells, whereas chemokines and other chemotactic factors mediate the recruitment and extravasion of selective cell populations to target tissues [3]. The chemokines are categorically divided into the CxC (or alpha) chemokines and the CC (or beta) chemokines. However, two recently discovered chemotactic proteins, lymphotactin and fractalkine, may warrant new familial designations as C and CxxxC chemokines, respectively. Although the chemokines were originally described as leukocyte chemoattractants, these small proteins have more recently been characterized as versatile mediators of acute and chronic inflammation because they function in angiogenesis, fibrogenesis, immune cell activation/regulation, and chemotaxis [4, 5]. Macrophage inflammatory protein 1α (MIP-1α) is a member of the CC chemokine family and is produced primarily by leukocytes, but not by stromal or structural cells. Once immune cells enter the target tissue or organ they continue to interact by cell-to-cell contact with structural cells. Earlier studies have demonstrated that monocyte/macrophage interactions with various cell populations can induce the production of chemokines [6]. These types of interactions may contribute to the persistent influx of leukocytes that characterizes chronic fibrotic diseases.

This study focuses on mechanisms by which cell-to-cell interactions may contribute to the pathological progression of chronic fibrotic diseases through specific interactions between an immune (macrophage) cell and a nonimmune stromal cell (fibroblast). These interactions appear to be mediated in part by ICAM-1-dependent adherence, which can lead to activation of a respiratory burst [7]. Furthermore, these oxygen radicals have been shown to induce chemokine production [8]. We found that in the absence of any pathogenic insult, the interaction between macrophages and fibroblasts, partially driven by the ICAM-1-mediated contact event, results in a significant increase in MIP-1α production. In addition, this chemokine release appears to be mediated via the generation of oxygen radical intermediates.

Abbreviations: MIP-1α, macrophage inflammatory protein 1α; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; TNF-α, tumor necrosis factor α; IL-1, interleukin-1; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; BSA, bovine serum albumin; IPF, idipathic pulmonary fibrosis.

Correspondence: Matthew L. Steinhauser, Department of Pathology, University of Michigan Medical School, 1301 Catherine Rd., Ann Arbor, MI 48109-0602. E-mail: matthewl@umich.edu.

Received March 20, 1998; revised July 13, 1998; accepted July 16, 1998.

MATERIALS AND METHODS

Animals

Specific pathogen-free CBA/J mice (6- to 12-week-old females, Charles River Breeding Labs) were used in all experiments requiring primary cell isolates. All mice were housed in specific pathogen-free conditions within the animal care facility at the University of Michigan (ULAM) until the day they were killed.

Peritoneal macrophage isolation

Mice were anesthetized and killed. The peritoneal cavity was exposed and approximately 10 mL of sterile 0.05 M EDTA solution was injected into the peritoneal cavity with a 26-gauge needle. A second needle was then inserted along a lateral side of the peritoneal cavity and the majority of the EDTA solution was withdrawn. This procedure was repeated approximately three times per mouse. On removal from the peritoneal cavity, the cell-containing EDTA solution was immediately placed on ice. The cells were pelleted by centrifugation at 1500 rpm after which the red blood cells were lysed using an ammonium chloride red blood cell lysing buffer. The cells were washed twice in RPMI, after which they were resuspended in 15% Dulbecco's modified Eagle's medium (DMEM).

Fibroblast isolation/culture

Lung fibroblasts were cultured from ICAM-1 knockout and wild-type CBA/J mice as previously described [9]. Briefly, whole lungs were removed from exsanguinated mice and transferred to RPMI 1640 containing 10% fetal bovine serum (FBS). The lung tissues were then ground into single cell preparations, after which red blood cells were lysed with an aluminum chloride-containing hypotonic lysing buffer for 2 min at 4°C. The remaining lung cells were then added to 175-mL tissue culture flasks. Cells were grown at 37°C in a humidified CO_2 incubator and fed DMEM containing 1% (v/v) antibiotic-antimycotic and 15% (v/v) FBS twice per week. After a minimum of two passages, homogenous fibroblast populations were transferred to six-well culture plates for experiments. Before experimental use, the fibroblast phenotype was confirmed in all cells by staining for α-actin and desmin in two-well Labtek® chamber culture slides. All fibroblasts were found to be completely free of α-naphthyl acetate esterase-positive macrophages.

Cell lines

Experiments to evaluate macrophage/fibroblast coculture-induced MIP-1α production were conducted with macrophage and fibroblast cell lines obtained from American Type Culture Collection (Rockville, MD). The murine fibroblasts cell line CL.7 (ATCC no. TIB 80) and the murine macrophage line RAW 264.7 (ATCC no. TIB 71) were cultured in DMEM containing 1% (v/v) antibiotic-antimycotic and 15% (v/v) FBS at 37°C in a humidified CO_2 incubator.

Reagents

Polyclonal tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and JE specific antiseras were generated in New Zealand White rabbits by multiple site immunization with recombinant murine cytokine/chemokine (R & D Systems, Minneapolis, MN) in complete Freund's adjuvant as previously described [10]. Polyclonal antibodies were titered by direct enzyme-linked immunosorbent assay (ELISA), and antibody specificity was verified for each ELISA.

Monoclonal antibodies to murine ICAM-1 (R & D Systems) and murine VCAM-1 (Chemicon International, Temecula, CA) were utilized in adhesion blocking experiments.

Murine cytokine ELISA

Murine MIP-1α was quantitated using a modification of a double-ligand method as previously described [11]. Briefly, flat-bottomed 96-well microtiter plates (Nunc Immuno-Plate I 96-F) were coated with 50 mL/well of rabbit antibody against the various cytokines (1 mg/mL in 0.6 M NaCl, 0.26 M H_3BO_4, and 0.08 M NaOH, pH 9.6) for 16 h at 4°C and then washed with phosphate-buffered saline (PBS), pH 7.5, 0.05% Tween-20 (wash buffer). Microtiter plate nonspecific binding sites were blocked with 2% bovine serum albumin (BSA) in PBS and incubated for 90 min at 37°C. Plates were rinsed four times with wash buffer and diluted (neat and 1:10) cell-free supernatants (50 mL) in duplicate were added, followed by incubation for 1 h at 37°C. Plates were washed four times, followed by the addition of 50 mL/well biotinylated rabbit antibodies against the specific cytokines (3.5 mg/mL in PBS, pH 7.5, 0.05% Tween-20, and 2% FCS), and plates incubated for 30 min at 37°C. Plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA) added, and the plates incubated for 30 min at 37°C. Plates were washed again four times and chromogen substrate (Bio-Rad Laboratories) added. The plates were incubated at room temperature to the desired extinction, and the reaction terminated with 50 mL/well of 3 M H_2SO_4 solution. Plates were read at 490 nm in an ELISA reader. Standards were 1/2 log dilutions of recombinant murine cytokines from 1 pg/mL to 100 ng/mL. This ELISA method consistently detected murine cytokine concentrations above 25 pg/mL. Antibody specificity was verified for each ELISA.

Statistical analysis

Data were evaluated by analysis of variance followed by two-tailed *t* test. All calculations were performed on a Power Macintosh 7200 computer, using Prism 2.0 (Graphpad Software, Inc., San Diego, CA). Significance was assigned for P values < 0.05.

RESULTS

MIP-1α production resulting from fibroblast/macrophage interaction

Prior studies have demonstrated that immune/non-immune cell interactions can induce chemokine production [11]. The primary intent of this study was to address whether this specific macrophage/fibroblast interaction was sufficient to induce the production of MIP-1α, a known inflammatory cytokine that is also chemotactic for monocytes. We first characterized the MIP-1α production associated with the addition of varying numbers of RAW cells to confluent CL7 fibroblast cultures (**Fig. 1**). The CL7/RAW cell cocultures produced a significant increase in MIP-1α production compared with control cultures. Furthermore, this increased production was clearly dependent on the number of RAW cells involved as we observed a threefold increase in production with 5×10^5 RAW cells versus 5×10^4 RAW cells. We also examined the kinetics of MIP-1α production in the CL7/RAW cell coculture system (**Fig. 2**). We found that increased MIP-1α secretion was already evident after 1 h of coculture. MIP-1α production peaked at 24 h, but was still prevalent at 48 h. Thus, we focused subsequent experiments on the 24-h time-point. It is interesting to note that CL7s appeared to specifically up-regulate MIP-1α expression. Of the chemokines examined, including MIP-1β, C10, MIP-2, KC, ENA-78, eotaxin, and JE, only MIP-1α production was significantly elevated in CL7/RAW cocultures.

ICAM-1 as a mediator of MIP-1α production

To determine the possible role of ICAM-1 in MIP-1α production resulting from the CL7/RAW cell interaction, purified ICAM-1-specific monoclonal antibodies were used to neutralize cell-surface expression of the adhesion molecule (**Fig. 3**). ICAM-1-specific antibodies significantly inhibited the MIP-1α production associated with the CL-7/RAW cell coculture, suggesting that ICAM-1 played a role in the production of MIP-1α during this cellular interaction. Furthermore, in a

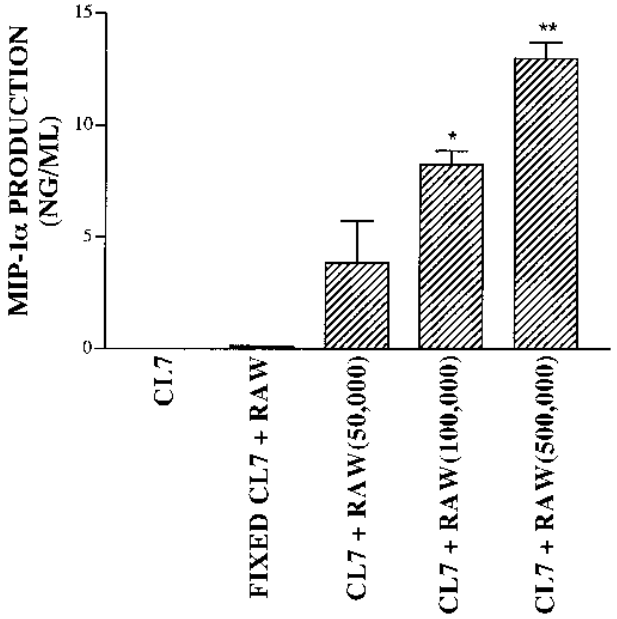

Fig. 1. Effect of varying numbers of RAW cells on CL7/RAW coculture-induced MIP-1α production. RAW cells were layered onto a CL7 monolayer. RAW cell controls were layered onto a fixed CL7 monolayer (10 min with 4% paraformaldehyde). MIP-1α concentrations were evaluated by ELISA after 24 h of incubation. Data expressed as mean + SE of one experiment ($n = 3$) representing two experiments with similar results. $^{*}P < 0.01$ or $^{**}P < 0.005$ as compared to fixed CL7 plus RAW control group.

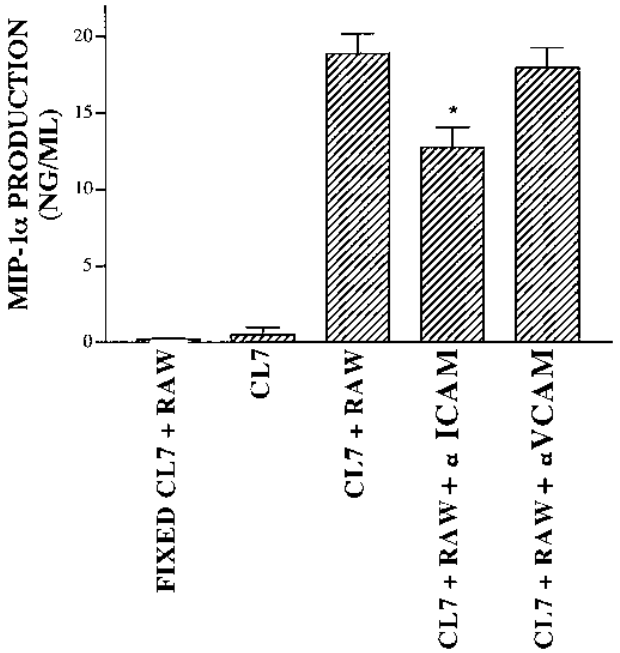

Fig. 3. Result of cell surface neutralization of ICAM-1 on MIP-1α production from CL7/RAW coculture. RAW cells (10^5/well) were layered onto a CL7 monolayer. RAW cell controls were layered onto a fixed CL7 monolayer (10 min with 4% paraformaldehyde). CL7/RAW cocultures were treated with monoclonal antibodies to ICAM-1 or VCAM-1. MIP-1α concentrations were evaluated by ELISA after 24 h of incubation and expressed as mean + SE. Experimental $n = 3$. However, this figure is representative of data from three similar experiments. $^{*}P < 0.05$ as compared to CL7 + RAW group.

primary cell isolate coculture system, consisting of peritoneal macrophages and lung fibroblasts isolated from ICAM-1 knockout mice, MIP-1α production was significantly decreased compared to a coculture system with control fibroblasts (**Fig. 4**). When control fibroblasts were separated from the peritoneal macrophage population by a transwell membrane (which allows the passage of soluble factors but prevents physical contact between the two cell populations), about 50% of coculture-induced MIP-1α production was prevented. These results suggest that ICAM-1 ligation clearly mediates a substantial portion of the adhesion-induced MIP-1α production. However, in the absence of ICAM-1 (as with the fibroblasts from ICAM-1 knockout mice), it appears that other events can also induce MIP-1α production.

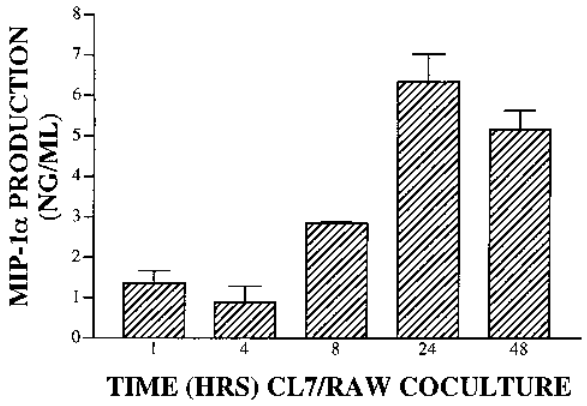

Fig. 2. Time-course of CL7/RAW coculture-induced MIP-1α production. RAW cells (10^5/well) were layered onto a CL7 monolayer. MIP-1α concentrations were evaluated by ELISA after 1, 4, 8, 24, and 48 h of incubation. Data expressed as mean + SE of one experiment ($n = 3$). Subsequent experiments displayed similar trends.

Macrophage/fibroblast coculture-induced MIP-1α production is not dependent on TNF-α, IL-1, or JE

TNF-α, IL-1, and JE have previously been implicated as regulators of chemokine production and adhesion molecule expression [12–14]. Thus, we explored the possibility that these cytokines might be directly or indirectly involved in the macrophage/fibroblast coculture-induced production of MIP-1α. Accordingly, each of these cytokines was neutralized with specific polyclonal rabbit antisera in the context of the CL7/RAW coculture. When compared to CL7/RAW cocultures treated with preimmune rabbit serum, none of the cytokine-specific antibody treatments significantly affected MIP-1α production (**Fig. 5**).

MIP-1α production and its dependence on oxygen radicals

Oxygen radicals have previously been identified to induce MIP-1α gene transcription [8], whereas adhesion to ICAM-1 can induce an oxidative burst in mononuclear phagocytes [7]. Thus, to deduce a possible role for oxygen radicals in the ICAM-1-induced production of MIP-1α, respiratory burst-mediated activation was neutralized with the nonspecific antioxidant *N*-acetylcysteine (NAC). In the CL7/RAW coculture system, MIP-1α production was reduced in a dose-dependent manner (**Fig. 6**). NAC reduced MIP-1α levels by

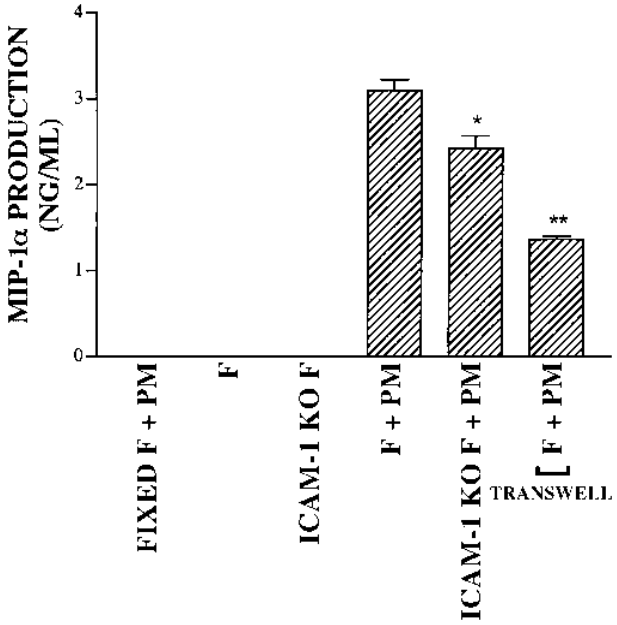

Fig. 4. Differential effects of normal fibroblasts versus ICAM-1 knockout fibroblasts (F) on peritoneal macrophage (PM)-derived MIP-1α, and the role of soluble and adhesive factors in the observed coculture-induced MIP-1α production. Peritoneal macrophages (10^5/well) were layered onto a fibroblast monolayer. PM controls were layered onto a fixed fibroblast monolayer (10 min with 4% paraformaldehyde). In the TRANSWELL group, the two cell populations were physically separated by a membrane that allows the passage of culture media plus any soluble factors. MIP-1α concentrations were evaluated by ELISA after 24 h of incubation and expressed as mean + SE. Data representative of two different experiments, experimental $n = 6$. $^*P < 0.01$, $^{**}P < 0.0001$ as compared to FIBRO + PM group.

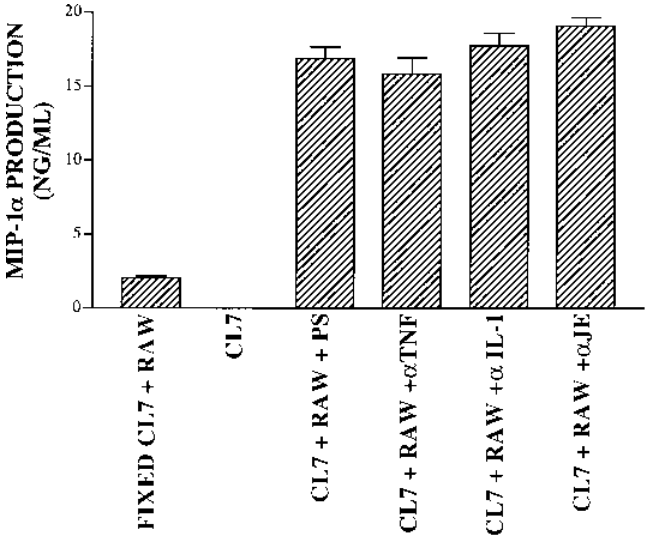

Fig. 5. The immunoneutralization of TNF-α, IL-1, and JE in the context of CL7/RAW cocultures, and the resultant effect on MIP-1α production. RAW cells (10^5/well) were layered onto a CL7 monolayer. RAW cell controls were layered onto a fixed CL7 monolayer (10 min with 4% paraformaldehyde). CL7/RAW cocultures were treated with preimmune serum (PS) or polyclonal antisera to TNF-α, IL-1, or JE. MIP-1α concentrations were evaluated by ELISA after 24 h of incubation and expressed as mean + SE. Experimental $n = 6$.

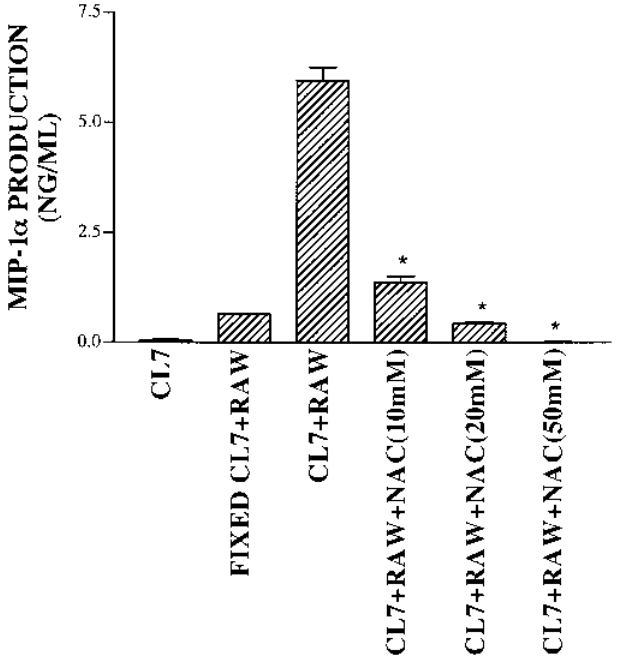

Fig. 6. Effect of *N*-acetylcysteine repression of macrophage super radical production on CL7/RAW coculture-induced MIP-1α levels. RAW cells (10^5/well) were layered onto a CL7 monolayer. RAW cell controls were layered onto a fixed CL7 monolayer (10 min with 4% paraformaldehyde). MIP-1α concentrations were evaluated by ELISA after 24 h of incubation and expressed as mean + SE. Data representative of two different experiments; experimental $n = 3$–6. $^*P < 0.0001$ as compared to CL7 + RAW group.

approximately 75 and 90% at 10 and 20 mM concentrations, respectively. At 50 mM, NAC completely abrogated MIP-1α production. Therefore, it appears that during fibroblast/macrophage interaction, oxygen radicals are necessary cofactors for MIP-1α production.

DISCUSSION

Chronic inflammatory and/or fibrotic disease results from a failure to resolve the immune response after the clearance of the inciting agent. The chronic disease state is characterized by several departures from the homeostatic environment of the tissue, including abnormal cytokine production, persistent infiltration of immune cells, and often an unnecessary deposition of matrix proteins [15]. This study explored one possible contributing mechanism by which chemokine production can become activated in the absence of any pathogenic challenge. Previously, we have shown that chemokine production could be induced by immune/non-immune cell interactions without any additional stimulus. Specifically, monocyte-endothelial cell interactions can induce the production of IL-8, MCP-1, and MIP-1α [11]. However, it has not been shown that immune/non-immune cell interactions can maintain chemokine production after the immune cell has entered the target tissue. We suspected that, as immune cells extravasate through the endothelium and infiltrate tissues, they can interact with other stromal cell types, resulting in elevated chemokine production. The specific cell-to-cell interaction between macrophage and fibroblast cell lines was chosen because macrophages represent

a major infiltrating immune cell population during chronic inflammatory disease and fibroblasts are commonly found dispersed throughout all tissue types, especially in and around fibrotic lesions. Fibroblast/macrophage coculture-induced MIP-1α production was initially found to depend on the number of macrophages, suggesting that MIP-1α production may be a self-perpetuating event, dependent on macrophage interactions with inflammatory fibroblasts. This may be an important issue because of this cytokine's role both as a chemotactic agent and as an immune activator. Specifically, MIP-1α has been described as a potent monocyte/macrophage chemoattractant, and as a macrophage activator, inducing the production of other inflammatory mediators such as IL-1 and TNF-α [16–18]. Furthermore, in the context of the fibrotic disease state, MIP-1α production has been associated with disease severity. For example, in both a murine model of bleomycin-induced pulmonary fibrosis and in human patients diagnosed with idiopathic pulmonary fibrosis (IPF), MIP-1α production was elevated when compared to control animals/patients [19]. In addition, in the bleomycin model, neutralization of MIP-1α attenuated disease severity [20]. Thus, chronic, self-perpetuation of MIP-1α production, as observed in the fibroblast/macrophage coculture system, presents a threat to host tissues, and is especially relevant in the context of fibrosis.

The progression of fibrotic disease also depends in part on the expression of various adhesion molecules because they are vital facilitators of immune cell extravasation through the endothelium [21]. However, we have also identified the possibility of adhesion molecule mediation of chemokine production during chronic inflammatory states. The β-integrins, for example, can mediate immune/non-immune cell adhesion events, spurring chemokine production [unpublished observations]. Furthermore, monocyte interaction with ICAM-1 (which binds β_2-integrins) on the surface of endothelial cells induced the expression of MIP-1α, whereas VCAM-1 seemed to have no role [11]. This study examined the role of both ICAM-1 and VCAM-1 adhesion molecules by using specific antibodies to neutralize their cell-surface expression. In this fibroblast/macrophage system, VCAM-1 appeared to have no role in the observed elevation in MIP-1α production, whereas the neutralization of ICAM-1 significantly inhibited MIP-1α production. In the coculture system, ICAM-1 knock out fibroblasts induced significantly less MIP-1α production compared with control fibroblasts. Although the reduction of MIP-1α was not as impressive as the anti-ICAM-1 data on the fibroblast cell line, it reiterated that ICAM-1 is an important adhesion molecule in the contact-induced production of MIP-1α. Although this study focused on the role of ICAM-1 in adhesion-induced chemokine expression, additional adhesion events probably participate in the up-regulation of MIP-1α production. CD44-mediated adhesion, for example, may account for some portion of the contact-induced MIP-1α production because it has previously been shown that CD44 binding of hyaluronan can result in MIP-1α production [22, 23]. It is interesting to note that the transwell membrane (which prevented the two cells' populations from physically interacting) only reduced MIP-1α production by 50% compared to fibroblast/macrophage coculture controls, suggesting that soluble factors in addition to adhesion molecules may also play a role in the induction of MIP-1α production. Furthermore, in the context of the CL7/RAW coculture system separated by transwell membrane, the addition of anti-ICAM-1 did not affect MIP-1α production, again suggesting an alternate mechanism of coculture-induced MIP-1α up-regulation (data not shown). In light of the possible role for soluble factors in the coculture-induced secretion of MIP-1α, we explored the possibility that a soluble cytokine signal was directly or indirectly responsible for the increased MIP-1α production. Several key proinflammatory cytokines, including TNF-α, IL-1, and JE, were immuno-neutralized in the context of CL7/RAW cocultures. However, none of the latter neutralizing conditions affected MIP-1α production compared to control cultures. Thus, because the proinflammatory cytokines IL-1, TNF-α, and JE do not appear to affect MIP-1α production, and because ICAM-1-mediated interactions cannot account for all of the coculture-induced MIP-1α production, other factors (i.e., interactions with matrix proteins, other adhesion molecules, or soluble factors) probably play a role in the observed levels of MIP-1α secretion.

Oxidants have been identified as important components of fibrotic disease because they not only directly damage tissues, but they additionally stimulate collagen production and the generation of fibrotic tissues [24]. This study examined oxygen radical participation in the stimulation of MIP-1α during macrophage/fibroblast interactions. It has previously been shown that cross-linking of ICAM-1 induces an oxidative burst from monocytes [7]. Furthermore, oxygen radicals have been identified as important effectors of chemokine production, including MIP-1α [8, 25]. In this study, the role of the respiratory burst in MIP-1α induction was characterized by inhibiting oxygen radical regulatory functions with the antioxidant NAC. In the macrophage/fibroblast coculture system, NAC suppressed MIP-1α production in a dose-dependent manner, completely neutralizing MIP-1α production at a 50 mM concentration. Furthermore, NAC effectively neutralized all MIP-1α production associated with CL7/RAW cocultures separated by a transwell membrane (data not shown), further implying that mechanisms of coculture induced MIP-1α production operating independently of ICAM-1 must ultimately depend on the elicitation of oxygen radicals. Thus, the respiratory burst appears to be a necessary cofactor for MIP-1α secretion in this macrophage/fibroblast coculture system, which may be pertinent to MIP-1α regulation, as our preliminary studies show that IL-1 and TNF-α are poor inducers of MIP-1α in monocytes compared to cell-to-cell interactions [unpublished data].

Overall, the results of this study indicate that during macrophage/fibroblast interactions substantial MIP-1α production is induced, and this event is dependent on ICAM-1-mediated ligation and oxidative burst activation. Although this mechanism by which a stromal cell activates immune cell-derived chemokine production could be relevant to various different inflammatory states, these data may be especially important during chronic disease because: (1) MIP-1α is a potent chemoattractant for macrophages, (2) MIP-1α functions as a macrophage activator, and (3) prolonged maintenance of these latter MIP-1α effects correlate with the pathogenesis of chronic diseases.

ACKNOWLEDGMENTS

This research was supported in part by grants from National Institues of Health (A136302 and HL35276) and the American Lung Association.

REFERENCES

1. Lukacs, N. W., Ward, P. A. (1996) Inflammatory mediators, cytokines, and adhesion molecules in pulmonary inflammation and injury. Adv. Immunol. 82, 257–304.
2. Kovacs, E. J. (1992) Fibrogenic cytokines: the role of immune mediators in the development of scar tissue. Immunol. Today 12, 17–23.
3. Springer, T. A. (1990) Adhesion receptors of the immune system. Nature 346, 425.
4. Mackay, C. R. (1997) Chemokines: what chemokine is that? Curr. Biol. 7, R384–R386.
5. Rollins, B. J. (1997) Chemokines. Blood 90, 909–928.
6. Lukacs, N. W., Strieter, R. M., Elner, V., Evanoff, H., Burdick, M. D., Kunkel, S. L. (1995) Production of chemokines, IL-8 and MCP-1, during monocyte:endothelial cell interactions is induced via matrix protein interactions. Blood 86, 2767–2773.
7. Rothlein, R., Kishimoto, T. K., Mainolfi, E. (1994) Cross-linking of ICAM-1 induces co-signaling of an oxidative burst from mononuclear leukocytes. J. Immunol. 152, 2488–2495.
8. Shi, M. M., Godleski, J. J., Paulauskis, J. D. (1996) Regulation of macrophage inflammatory protein-1alpha mRNA by oxidative stress. J. Biol. Chem. 271, 5878–5883.
9. Lukacs, N. W., Chensue, S. W., Smith, R. E., Strieter, R. M., Warmington, K., Wilke, C., Kunkel, S. L. (1994) Production of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 by inflammatory granuloma fibroblasts. Am. J. Pathol. 144, 711–718.
10. Evanoff, H., Burdick, M., Moore, S., Kunkel, S., Strieter, R. (1992) A sensitive ELISA for the detection of human monocyte chemoattractant protein-1 (MCP-1). Immunol. Invest. 21, 39–45.
11. Lukacs, N. W., Strieter, R. M., Elner, V. M., Evanoff, H. L., Burdick, M. D., Kunkel, S. L. (1994) Intracellular adhesion molecule-1 mediates the expression of monocyte-derived MIP-1α during monocyte-endothelial cell interactions. Blood 83, 1174–1178.
12. Muhl, H., Dinarello, C. A. (1997) Macrophage inflammatory protein-1 alpha production in lipopolysaccharide-stimulated human adherent blood mononuclear cells is inhibited by the nitric oxide synthase inhibitor *N*(G)-monomethyl-L-arginine. J. Immunol. 159, 5063–5069.
13. Cheshire, J. L., Baldwin, A. S., Jr. (1997) Synergistic activation of NF-kappaB by tumor necrosis factor alpha and gamma interferon via enhanced I kappaB alpha degradation and de novo I kappaBbeta degradation. Mol. Cell. Biol. 17, 6746–6754.
14. Goebeler, M., Yoshimura, T., Toksoy, A., Ritter, U., Brocker, E. B., Gillitzer, R. (1997) The chemokine repertoire of human dermal microvascular endothelial cells and its regulation by inflammatory cytokines. J. Invest. Dermatol. 108, 445–451.
15. Zhang, K., Phan, S. H. (1996) Cytokines and pulmonary fibrosis. Biol. Signals 5, 232–239.
16. Driscoll, K. E. (1994) Macrophage inflammatory proteins: biology and role in pulmonary inflammation. Exp. Lung Res. 20, 473–490.
17. Smith, R. E., Strieter, R. M., Zhang, K., Phan, S. H., Standiford, T. J., Lukacs, N. W., Kunkel, S. L. (1995) A role for C-C chemokines in fibrotic lung disease. J. Leukoc. Biol. 57, 782–787.
18. Wolpe, S. D., Davatelis, G., Sherry, B., Beutler, B., Hesse, D., Nguyen, H. T., Molawer, L. L., Nathan, C. F., Lowry, S. F., Cerami, A. (1988) Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. J. Exp. Med. 167, 570–581.
19. Standiford, T. J., Rolfe, M. W., Kunkel, S. L., Lynch, J. P., Burdick, M., Gilbert, A. R., Orringer, M. B., Whyte, R. I., Strieter, R. M. (1993) Macrophage inflammatory protein-1alpha expression in interstitial lung disease. J. Immunol. 151, 2852–2863.
20. Smith, R. E., Strieter, R. M., Phan, S. H., Lukacs, N. W., Huffnagle, G. B., Wilke, C. A., Burdick, M. D., Lincoln, P., Evanoff, H., Kunkel, S. L. (1994) Production and function of murine macrophage inflammatory protein-1 alpha in bleomycin-induced lung injury. J. Immunol. 153, 4704–4712.
21. Demling, R. H. (1995) The modern version of adult respiratory distress syndrome. Annu. Rev. Med. 46, 193–202.
22. McKee, C., Penno, M., Cowman, M., Burdick, M., Strieter, R., Bao, C., Noble, P. (1996) Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages. The role of HA size and CD44. J. Clin. Invest. 98, 2403–2413.
23. Horton, M., Burdick, M., Strieter, R., Bao, C., Noble, P. (1998) Regulation of hyaluronan-induced chemokine gene expression by IL-10 and IFN-gamma in mouse macrophages. J. Immunol. 160, 3023–3030.
24. Freeman, B. A., Crapo, J. D. (1982) Biology of disease. Free radicals and tissue injury. Lab. Invest. 47, 412–426.
25. VanOtteren, G. M., Standiford, T. J., Kunkel, S. L., Danforth, J. M., Strieter, R. M. (1995) Alterations of ambient oxygen tension modulate the expression of tumor necrosis factor and macrophage inflammatory protein-1 alpha from murine alveolar macrophages. Am. J. Respir. Cell Mol. Biol. 13, 399–409.