Cell-to-cell and cell-to-matrix interactions mediate chemokine expression: an important component of the inflammatory lesion

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Abstract: Although many studies have characterized soluble factors that stimulate or inhibit chemokine secretion, in this review we focus on the event of cellular adhesion as a novel mechanism for stimulating chemokine expression. Recent work has demonstrated chemokine expression following cell-to-cell and cell-to-matrix adhesion. The specificity of this finding was demonstrated utilizing various techniques that illustrate that adhesion, and not a soluble stimulus, is in some cases responsible for initiating or augmenting chemokine expression. For example, co-cultures of peripheral blood monocytes and endothelial cells secreted elevated levels of IL-8 and MCP-1 compared with either cell type alone. When co-cultured in transwells, this effect was significantly attenuated. In other experiments, neutralizing monoclonal antibodies to various adhesion molecules inhibited chemokine expression. The effects of adhesion were not limited to leukocytes. Both immune and non-immune cell types were evaluated as potential sources of adhesion-mediated chemokine expression. Not suprisingly, expression of some chemokines was associated with adhesion, whereas others were not, supporting the notion that adhesion differentially signals chemokine secretion during the inflammatory response. We hypothesize that as a recruited leukocyte encounters different adhesion substrates such as endothelial cells, basement membrane, extracellular matrix, and fibroblasts, the expression of chemokines from both the leukocyte and the substrate may be initiated, inhibited, or augmented. Careful characterization of the contribution of adhesion to regulation of chemokine expression will provide insight into the pathogenesis of many human diseases where chemokines have a central role. *J. Leukoc. Biol.* 62: 612–619; 1997.

Key Words: *cellular adhesion · transwells · interleukin-8 · monocyte chemoattractant protein-1*

INTRODUCTION

Chemotactic cytokines, or chemokines, have been identified as important mediators of the multicomponent lesion generated during an inflammatory response. In addition, chemokines contribute to diverse biological functions, including angiogenesis, hematopoesis, embryogenesis, and endogenous pyrogenesis [1–11]. After their discovery, many chemokines were implicated as direct mediators of pathogenesis in animal models of diseases such as asthma, idiopathic pulmonary fibrosis (IPF), sepsis, inflammatory bowel disease, multiple sclerosis, and bacterial pneumonia [12–19]. Thus, characterization of the processes that regulate chemokine secretion will likely yield significant insights for the development of novel treatment modalities for many human diseases. Current studies have addressed mechanisms responsible for activating chemokine secretion. At present, the following two primary mechanisms for chemokine stimulation have been described: (1) stimulation of a target cell via soluble pro-inflammatory mediators such as tumor necrosis factor (TNF) or interleukin-1 (IL-1) and (2), more recently characterized, stimulation via direct cell-to-cell interaction, mediated via adhesion molecules. In this article, we will discuss recent investigations into the role of the cellular adhesion event as a means for generating and regulating chemokine expression in various in vitro models of inflammation.

Chemokines are low-molecular-weight proteins that were recently cloned and characterized, yielding many putative mediators of macrophage, lymphocyte, mast cell, and granulocyte-derived responses in disease models [20, 21]. A large number of the described chemokines can be grouped into one of two families based on the juxtaposition of two cysteine residues at the amino terminus. The so-called C-C chemokine family includes macrophage inflammatory protein-1α (MIP-1α), MIP-1β, RANTES,

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Abbreviations: IPF, idiopathic pulmonary fibrosis; TNF, tumor necrosis factor; IL-1, interleukin-1; MIP-1α, macrophage inflammatory protein-1α; MCP-1, monocyte chemoattractant protein-1; IFN-γ, interferon-γ; IP-10, IFN-γ-inducible protein-10; PBM, peripheral blood monocytes; LPS, lipopolysaccharide; MLR, mixed lymphocyte reaction; HUVECs, human umbilical vein endothelial cells; SCF, stem cell factor; SOD, superoxide dismutase.

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Received June 25, 1997; revised August 21, 1997; accepted August 22, 1997.

monocyte chemoattractant protein-1 (MCP-1), MCP-2, MCP-3, MCP-4, MCP-5, C10, eotaxin, and I-309. C-C chemokines were originally described as chemotactic factors but are also known to modulate cytokine production, adhesion molecule expression, and mononuclear cell proliferation [15, 22–24]. Members of the C-X-C chemokine family were originally characterized as potent neutrophil chemotactic and activating factors. Members of the C-X-C family include IL-8, interferon-γ (IFN-γ) -inducible protein-10 (IP-10), neutrophil activating protein-2 (NAP-2), murine KC, murine MIP-2, ENA-78, and others [25]. Recently, other functions for C-X-C chemokines have been elucidated, and include effects on angiogenesis as well as lymphocyte chemotaxis and activation [26, 27]. C-C and C-X-C chemokines are secreted in a stimulus-specific manner by cytokines from all types of leukocytes, fibroblasts, glial cells, smooth muscle cells, endothelial cells, and epithelial cells [14, 28–35]. Although much is known regarding the expression of chemokines from the aforementioned cell types, few studies have evaluated the role of intimate cell-to-cell and cell-to-matrix interactions as a stimulus for chemokine expression.

ADHESION MOLECULES MEDIATE LEUKOCYTE RECRUITMENT

Cell-to-cell interactions take on great significance during inflammation, particularly since resident or recruited leukocytes are intimately associated with extracellular matrix proteins, stromal cells, and/or cells of the inflamed organ. These cell-to-cell and cell-to-matrix interactions are mediated by adhesion molecules. Adhesion molecules are a group of heterogeneous cell surface receptors that provide the means for mobility and trafficking of leukocytes through the complex biological architecture of tissues and organs [36]. Both recirculation and recruitment of a leukocyte require the cell to extravasate from the intravascular space, a process mediated by an elegant sequence of adhesion molecule interactions. During the generation of inflammatory responses, leukocytes are recruited to the site of an inflammatory insult via interactions between adhesion molecules and their substrates. First, leukocytes marginate or roll along the vascular endothelium mediated by a reversible, relatively high K_d interaction between selectins and their counter-receptors. In the absence of a secondary signal, the leukocyte may detach or continue to roll, but does not stop. In the presence of a stimulus, such as TNF or IL-1, additional adhesion molecules are translocated to the endothelial cell surface, which facilitates stopping of the leukocyte with a much stronger interaction, allowing the subsequent events of diapedesis and chemotaxis of the leukocyte into a site of an inflammatory insult [36]. The movement of specific populations of leukocytes into tissues is probably mediated by concentration gradients of chemokines and other chemotactic factors. Once through the endothelial cell layer, the leukocyte may interact with many different extracellular matrix proteins such as fibronectin, laminin, fibrinogen, collagen, and thrombospondin. In addition, the recruited leukocyte may interact directly with other leukocytes, as well as stromal cells such as fibroblasts or smooth muscle cells. The interaction of the leukocyte with the extracellular matrix and other cells is a dynamic process whereby the recruited leukocyte expresses different adhesion molecules and secretes cytokines in a stimulus-specific manner. Thus, as the leukocyte moves through the vessel wall into the interstitial space, it is responding to new tissue-specific stimuli and actively secreting inflammatory substances.

ADHESION MOLECULES STIMULATE CHEMOKINE EXPRESSION

Initial investigations of adhesion phenomena, which preceded the discovery of chemokines, demonstrated adhesion-dependent expression of pro-inflammatory cytokines from leukocytes. These in vitro studies demonstrated expression of TNF and IL-1 from peripheral blood monocytes (PBM) following adhesion to plastic and/or various extracellular matrix proteins [37–41]. More recently, using a similar approach, chemokine gene expression was detected by evaluating a cDNA library made from peripheral blood monocytes stimulated by adherence to plastic for 30 min. Up-regulation of mRNAs for IL-8, a C-X-C chemokine, as well as other transcripts with homology to other chemokines was detected [42]. Consistent with these findings, adherence to plastic plates stimulated both IL-8 mRNA and protein from peripheral blood mononuclear cells compared with cells incubated in Teflon chambers [43]. IL-8 mRNA expression peaked at 8 h, whereas IL-8 protein peaked at 24 h. The addition of cycloheximide, but not anti-TNF or anti-IL-1 neutralizing antibodies, attenuated IL-8 mRNA expression after adhesion to plastic plates [43]. This result suggests that de novo expression of a protein other than IL-1 or TNF is necessary for adherence-mediated IL-8 mRNA expression. However, while not necessary for IL-8 expression, addition of exogenous lipopolysaccharide (LPS), IL-1, or TNF potentiated IL-8 mRNA and protein expression after adherence to plastic [44]. In related experiments, IL-8 expression was also induced after monocyte adherence to collagen- or fibronectin-coated culture plates [45]. Similar to IL-8, the murine C-X-C chemokines MIP-2 and KC were expressed after adherence of murine alveolar or peritoneal macrophages to plastic [R. E. Smith, N. W. Lukacs, H. Evanoff, M. D. Glass, R. M. Strieter, and S. L. Kunkel, unpublished results]. IL-10 inhibited expression of these two chemokines in a dose-dependent manner. The expression of potent chemotactic factors like IL-8, MIP-2, and KC after adhesion of monocytes to plastic lends insight to events that may be occurring in vivo. As a freshly recruited monocyte enters the inflammatory lesion via adhesion molecule interactions, IL-8 secretion would amplify the inflamma-

tory process by stimulating chemotaxis of additional leukocytes. In addition, the presence of TNF, IL-1, and LPS, substances found at sites of bacterial infection, could potentiate IL-8 secretion from the adherent monocyte, further increasing leukocyte accumulation.

ADHESION-MEDIATED CHEMOKINE EXPRESSION FROM A MIXED LYMPHOCYTE REACTION

The mixed lymphocyte reaction (MLR) is an in vitro model of delayed-type hypersensitivity and allograft rejection that has provided insights into the mechanisms of T cell activation and proliferation. Several cytokines have been identified as mediators of the alloantigen-driven T cell proliferative responses observed in the MLR. TNF and IL-2 were identified as partial mediators of this response supported by the evidence that neutralization of either IL-2 or TNF attenuated lymphocyte proliferation [46–48]. Not surprisingly, chemokine expression was detected in supernatants from the MLR. IL-8 and MCP-1 protein and mRNA were elevated at 24 h post-initiation of the mixed lymphocyte response, and immunohistochemical localization identified mononuclear phagocytes as a source of these chemokines [49]. Neutralization of IL-8 or MCP-1 with polyclonal antibodies did not affect lymphocyte proliferation. However, neutralization of TNF did attenuate MCP-1 and IL-8 protein expression [49]. These findings suggest that TNF is the primary stimulant for chemokine expression in the MLR. However, a subsequent study demonstrated otherwise. Addition of anti-ICAM-1 and anti-LFA-3 antibodies to the MLR significantly decreased proliferation as well as expression of TNF, IL-8, MCP-1, and MIP-1α protein [50]. To demonstrate that a mechanism of chemokine stimulation other than that initiated by TNF was present, the MLR was reconstituted with exogenous TNF or IL-2. Reconstitution did not restore MCP-1 or MIP-1α, but did restore IL-8 expression, suggesting that adhesion molecules act differentially as stimuli for chemokine expression when appropriate cell types interact [50]. IL-8 expression may be regulated in the MLR by either adhesion molecule interactions or a cytokine cascade involving TNF. In contrast, expression of MCP-1 and MIP-1α required both soluble and adhesion molecule-mediated signals.

In the above MLR experiments addition of anti-ICAM-1 antibodies attenuated IL-8, MIP-1α, and MCP-1 expression at 1 h and at 4 days post-initiation of the MLR. However, at 24 h post-initiation, treatment with anti-ICAM-1 antibodies increased MCP-1 and IL-8, but not MIP-1α, protein expression [50]. One hypothesis that may account for this finding is that the anti-ICAM-1 antibody is stimulating, rather than neutralizing, the signal mediated by the adhesion event, similar to anti-CD-3 antibody-mediated lymphocyte activation. Currently there is no other evidence supporting the notion that ICAM-1 is acting like CD-3. An alternative hypothesis is that this observation is due to activation of monocyte Fc-receptors with the Fc portion of the anti-ICAM antibody, stimulating chemokine expression. Consistent with this hypothesis, immobilized IgG and IL-1 strongly synergized to induce expression of I-309, another C-C chemokine, from PBMs [51]. These findings suggest that immune complex deposition may be a potent stimulus for chemokine expression, possibly contributing to significant pathology in diseases with immune complex deposition.

MONONUCLEAR CELL ADHESION TO ENDOTHELIAL CELLS STIMULATES CHEMOKINE EXPRESSION

Although the MLR involves only leukocytes, a co-culture system employing a monolayer of endothelial cells plus various types of purified leukocytes has yielded interesting results. Elevated levels of IL-8, MCP-1, and MIP-1α protein and mRNA were detected after placement of adherence-purified monocytes on human umbilical vein endothelial cells (HUVECs) primed with IFN-γ [33, 52, 53]. Unlike the monocytes-HUVEC co-cultures, placement of an enriched population of unstimulated lymphocytes on a HU VEC monolayer did not increase IL-8, MCP-1, or MIP-1α expression [33, 52]. Anti-ICAM-1-, but not anti-VCAM-1-, antibodies inhibited monocyte-endothelial cell co-culture-derived MIP-1α protein expression [52]. In contrast, anti-ICAM-1 antibodies had no effect on expression of MCP-1 or IL-8 from these cultures [33]. However, preincubation of HUVECs and monocytes before co-culturing with soluble type-1 collagen or separation of the HUVECs and monocytes into transwell co-cultures [transwell (Corning Costar Corp., Cambridge, MA) co-cultures physically separate two different populations of cells but allow exchange of soluble factors secreted by each population from one chamber to another] significantly attenuated both IL-8 and MCP-1 expression [33]. In addition, anti-TNF and anti-IL-1 antibodies did not inhibit expression of IL-8 and MCP-1 from the HUVEC-monocyte co-culture. These results suggest that, like MIP-1α, expression of MCP-1 and IL-8 is mediated, at least in part, by adhesion molecule interactions. It is interesting to note that the HUVEC monolayer was the major source of the elevated MCP-1, whereas both HU VECS and monocytes were a source for IL-8, and MIP-1α expression was predominately from monocytes [33, 52]. In contrast with monocytes, granulocyte-HUVEC co-cultures expressed elevated levels of MIP-1α but not MCP-1 protein, suggesting that chemokine expression after adhesion to endothelial cells is dependent on the type of leukocyte. In summary, these findings support the hypothesis that expression of chemokines is differentially modulated from both cellular participants via specific adhesion molecule-substrate interactions.

Lymphocytes-HUVEC co-cultures exhibited different regulation of MCP-1 and MIP-1α expression compared with monocyte-HUVEC co-cultures. Lymphocytes alone

activated with concanavalin A (conA) secreted elevated levels of MIP-1α and MCP-1, although MIP-1α protein expression was about twofold greater than MCP-1 expression. However, HUVECs co-cultured with conA-stimulated lymphocytes had significantly decreased (90% decrease) MIP-1α expression and significantly increased MCP-1 expression (125% increase) [unpublished observations]. Subsequent experiments demonstrated that co-culturing conA-activated lymphocytes with HUVECs abrogates lymphocyte-derived TNF, IFN-γ, and IL-12 expression [unpublished observations]. These results suggest that endothelial cell-derived, adhesion-mediated expression of MCP-1 is inhibiting lymphokine expression. This result is inconsistent with the current paradigm that lymphocytes, but not stromal cells, are the primary modulator of the inflammatory lesion.

CHEMOKINE EXPRESSION FROM LEUKOCYTE-FIBROBLAST CO-CULTURES

Recent investigations in our laboratory have focused on the role of the fibroblast during the generation and maintenance of the inflammatory lesion. Monocyte-fibroblast co-cultures spontaneously expressed elevated levels of the C-C chemokines MIP-1α and MCP-1 compared with cultures of either cell type alone. Neutralizing antibodies specific for β_3 integrin, but not antibodies specific for β_2 integrin, β_1 integrin, or ICAM-1, significantly attenuated MIP-1α protein expression from the monocyte-fibroblast co-culture [C. Zickus, S. L. Kunkel, H. Evanoff, M. D. Glass, R. M. Strieter, and N. W. Lukacs, unpublished results]. Dissimilar to MIP-1α, MCP-1 expression from these co-cultures was not inhibited by antibodies specific for β_3 integrin. In addition, antibodies specific for β_2 integrin, β_1 integrin, ICAM-1, VCAM-1, and E-selectin had no effect on MCP-1 expression, whereas anti-TNF antibodies did attenuate MCP-1 expression from the co-culture [C. Zickus, S. L. Kunkel, H. Evanoff, M. D. Glass, R. M. Strieter, and N. W. Lukacs, unpublished results]. Co-culture experiments utilizing either glutaraldehyde-fixed monocytes or fibroblasts have identified the cellular sources of MIP-1α and MCP-1. Similar to the HUVEC-monocyte co-culture experiments, the monocyte was the predominant source of MIP-1α expression, whereas the fibroblast was the predominant source of the MCP-1 expression [C. Zickus, S. L. Kunkel, H. Evanoff, M. D. Glass, R. M. Strieter, and N. W. Lukacs, unpublished results].

In a related study, CL-7-fibroblast-Raw 264.7-macrophage co-cultures expressed elevated levels of eotaxin, a C-C chemokine with potent eosinophil chemotaxis activity in vitro. Expression of eotaxin was abrogated when the two cell populations were placed in transwell cultures, demonstrating the requirement of cell-to-cell contact for the observed effect [N. W. Lukacs, personal communication]. These findings demonstrate that the fibroblast is not a passive bystander during the response to an inflammatory insult and is directly involved in the regulation of chemokine expression.

The monocyte is just one of many types of leukocytes actively recruited to the inflammatory lesion. Like monocytes, mast cells have been implicated as mediators of various disorders. In particular, the involvement of mast cells in reactive airway disease has been well documented. Histological localization revealed that peribronchial mast cells are in close proximity to fibroblasts and smooth muscle cells [54]. Co-cultures of mast cells and fibroblasts secreted elevated levels of MIP-1β, MCP-1, eotaxin, and C10, all of which are C-C chemokines with potent leukocyte activating and chemotactic activities. In addition, expression of eotaxin was potentiated when TNF was added to the co-culture, whereas its expression was attenuated by either placement of the co-cultures in transwells or treatment with anti-stem cell factor (SCF) antibodies [N. W.

TABLE 1. Adhesion Molecule-Mediated Chemokine Expression From Leukocytes on Plastic or Co-cultures with Leukocytes and Extracellular Matrix, Fibroblast, or Endothelial Cells

Type of co-culture cell/cell or cell/matrix	Adhesion molecules or receptors involved[a]	Chemokine expressed	Soluble co-stimulus[a]
PBM/plastic	β_2 integrin	IL-8	Unknown
Alveolar macrophages/plastic	β_2 integrin	MIP-2	Unknown
Peritoneal macrophages/plastic	β_2 integrin	MIP-2	Unknown
Mixed lymphocyte reaction	ICAM-1, LFA-3	IL-8	TNF?
Mixed lymphocyte reaction	ICAM-1, LFA-3	MCP-1	TNF
PBMs/immobilized IgG	Fc receptor	I-309	IL-1
PBMs/collagen or fibronectin	Unknown	IL-8	TNF, IL-1
PBMs/HUVEC	Unknown	IL-8, MCP-1	None
PBMs/HUVEC	ICAM-1	MIP-1α	None
Granulocyte/HUVEC	Unknown	MIP-1α	Unknown
Lymphocytes (ConA)/HUVEC	Unknown	MCP-1, MIP-1α	Unknown
PBMs/16-Lu fibroblasts	β_3 integrin	MIP-1α	None
RAW 264.7/CL-7 fibroblasts	Transmembrane SCF?	Eotaxin	Unknown
Mast cells/lung fibroblasts	Unknown	MIP-2	Unknown

[a] Unknown indicates not investigated; none indicates transwell or neutralization experiments abrogate expression of chemokine.

Lukacs, R. M. Strieter, C. Hogaboam, P. Lincoln, D. D. Taub, T. J. Standiford, and S. L. Kunkel, unpublished results]. These results support the hypothesis that a transmembrane form of SCF, a potent mast cell activator, is mediating eotaxin expression from the co-culture. The mast cell-fibroblast interaction also appears to mediate differential expression of C-X-C chemokines. MIP-2, but not KC, was secreted in a contact-dependent fashion from the mast cell fibroblast co-cultures [unpublished observations]. In contrast to eotaxin, MIP-2 expression was not attenuated by anti-SCF antibodies, suggesting differential pathways for activation of chemokine expression. Studies are underway to identify the cellular sources of the various chemokines secreted by these cultures. Regardless of the cellular source, adhesion-dependent expression of chemokines following mast cell-fibroblast interactions probably contributes significantly to the pathology of mast cell-related disease.

Numerous recent studies suggest that MCP-1 exerts distinct immunomodulatory effects that appear to extend beyond its role in chemoattraction of mononuclear cells to sites of inflammation [55–57]. For example, endogenous MCP-1 production by tissue structural cells such as lung fibroblasts and smooth muscle cells, which greatly surpasses production of this mediator by numerous inflammatory cells, including macrophages, is important in the modulation of T cell cytokine production and proliferation [58 and unpublished results]. In co-culture experiments similar to those described above, the constitutive and cytokine-stimulated generation of MCP-1 by lung fibroblasts was necessary for de novo T cell-derived IL-4 expression after T cell-fibroblast adhesion. This effect was demonstrated through both specific immunoneutralization of MCP-1 protein using a polyclonal antibody and selective degradation of MCP-1 mRNA transcripts with MCP-1 mRNA-specific antisense oligonucleotides. The inhibition of MCP-1 expression during lung fibroblast-T cell co-culture promoted IFN-γ production by the T cells. Another function for the generation of MCP-1 by structural tissues may be as a protective mechanism to ensure that during interactions between fibroblasts and T cells, cytokines are generated that have immunomodulatory rather than proinflammatory roles. This is borne out by recent evidence that in the absence of MCP-1 production by fibroblasts, T cells have a propensity to proliferate during cell-to-cell interactions with lung fibroblasts. This form of regulation of T cell cytokine expression by stromal cells suggests a bidirectional interaction between immune and nonimmune cells that relies on immunomodulatory proteins, a notion inconsistent with the current paradigm of T cell regulation. In summary, these data support a role for chemoattractant proteins such as MCP-1 in the dynamic interaction between immune and non-immune cells, and also suggest that manipulating the production of stromal cell-derived chemokines may have therapeutic potential in inflammatory conditions.

PUTATIVE MECHANISMS OF ADHESION-MEDIATED CHEMOKINE EXPRESSION

Because chemokines have previously been shown to promote adhesion events, it is technically challenging to elucidate which comes first, cellular adhesion or chemokine expression. Nevertheless, the data presented in this review strongly support the notion that in some circumstances adhesion precedes, and likely stimulates, chemokine expression. Although the signal transduction pathways that link adhesion with chemokine expression have not been specifically elucidated, several recent studies have described novel components of the signaling pathways likely involved. Engagement of ICAM-1 on a rheumatoid synovial cell line induced activation of the transcription factor AP-1 and concurrently stimulated IL-1 mRNA expression [59]. Because IL-1 and chemokines may have similar signaling pathways, this study suggests that AP-1 may participate in the connection between adhesion and chemokine secretion. In other studies, VLA-4, VLA-5, LFA-1, and ICAM-1 all have been shown to mediate activation of protein tyrosine kinases [60, 61]. Consistent with these findings, megakaryocyte adhesion to fibronectin induced localization of a novel adhesion focal tyrosine kinase to focal adhesion-like structures, and integrin-mediated cell adhesion signaled tyrosine phosphorylation of intracellular proteins [62]. The initiation of these signaling processes following adhesion events similar to those found in inflammatory lesions may play a role in mediating chemokine expression.

Although the details of adhesion-mediated signaling are unclear, other evidence suggests that the respiratory burst may have a role in adhesion-mediated chemokine expression. Recent studies have described expression of MIP-1α, IL-8, and an unidentified small-molecular-weight protein with chemotactic activity from leukocytes following exposure to reactive oxygen species [63–65]. In addition, numerous other studies have demonstrated that the respiratory burst is dependent on leukocyte adhesion plus a soluble co-stimulus, such as TNF, IL-1, or bacterial products [66, 67]. Based on these findings, we propose that adhesion-dependent chemokine expression may be initiated, or at the least augmented, via the leukocyte respiratory burst. Supporting this hypothesis, expression of MIP-1α and IL-8 from HUVEC-monocyte co-cultures was attenuated by preincubation of each cell population for 4 h with superoxide dismutase (SOD; **Fig. 1**). Preincubation with SOD had no effect on MCP-1 expression from the co-culture. Because co-culture expression of MCP-1 is probably from the endothelial cell, it may be that the effects of reactive oxygen species on chemokine expression are limited to leukocytes or specific chemokines. These data support the notion that the oxidative burst, at minimum, augments adhesion-mediated chemokine expression and is probably one of many important stimuli of chemokine secretion during the inflammatory response.

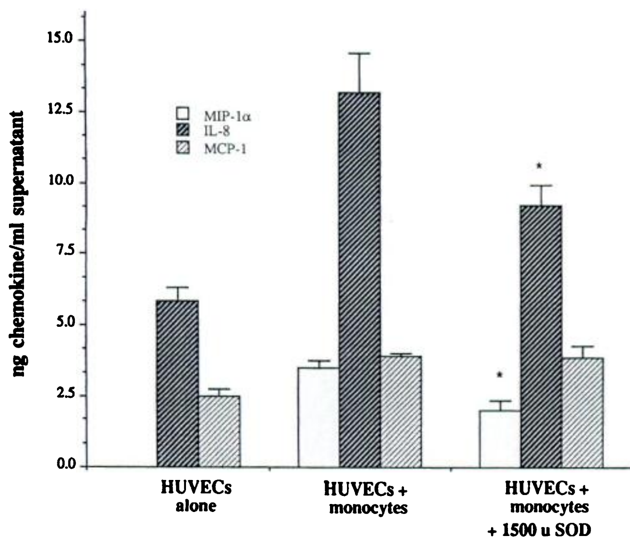

Fig. 1. MIP-1α, MCP-1, and IL-8 enzyme-linked immunosorbent assay analysis of culture supernatants from HUVECs alone, HUVECS plus monocytes, and HUVECS plus monocytes pretreated with 1500 units of SOD ($n = 6$, $*P < 0.05$).

SUMMARY AND CONCLUSIONS

Although the mechanism of adhesion-mediated chemokine expression is not yet determined, the adhesion event is a potent stimulus for chemokine expression (**Table 1**). This pathway for the expression of chemokines probably has a central role in vivo during the generation of the inflammatory lesion. For example, the response to an inflammatory insult involves expression of TNF and IL-1, cytokines that would activate adjacent endothelium, interstitial fibroblasts, resident leukocytes, and/or cells of the inflamed organ (**Fig. 2**). Next, the inflamed tissues would probably secrete various lipid and protein chemotatic factors, directing chemotaxis and the recruitment of various leukocytes, such as monocytes, to the lesion. During this response, adhesion of recruited monocytes to endothelial cells (Fig. 2A), extracellular matrix (Fig. 2B), or fibroblasts (Fig. 2C) mediated via ICAM-1, β_2 integrins, or β_3 integrins, respectively, could stimulate expression of proinflammatory molecules from the monocyte such as MCP-1, MIP-1α, or IL-8. Expression of these chemokines in or adjacent to the lesion would amplify the inflammatory response by contributing to the recruitment, retention, and activation of additional leukocytes.

Some inflammatory lesions do not resolve, progressing to chronicity. Chemokine expression from lymphocytes or macrophages that are intimately localized with stromal cells in a chronic inflammatory lesion is, in part, probably mediated via adhesion. Such processes would provide a potent, localized signal that might help maintain the chronic lesion. Consistent with this notion, expression of receptors for MCP-1, MIP-1α, and IL-8, chemokines secreted by stromal cells, has been demonstrated on specific subsets of leuko-

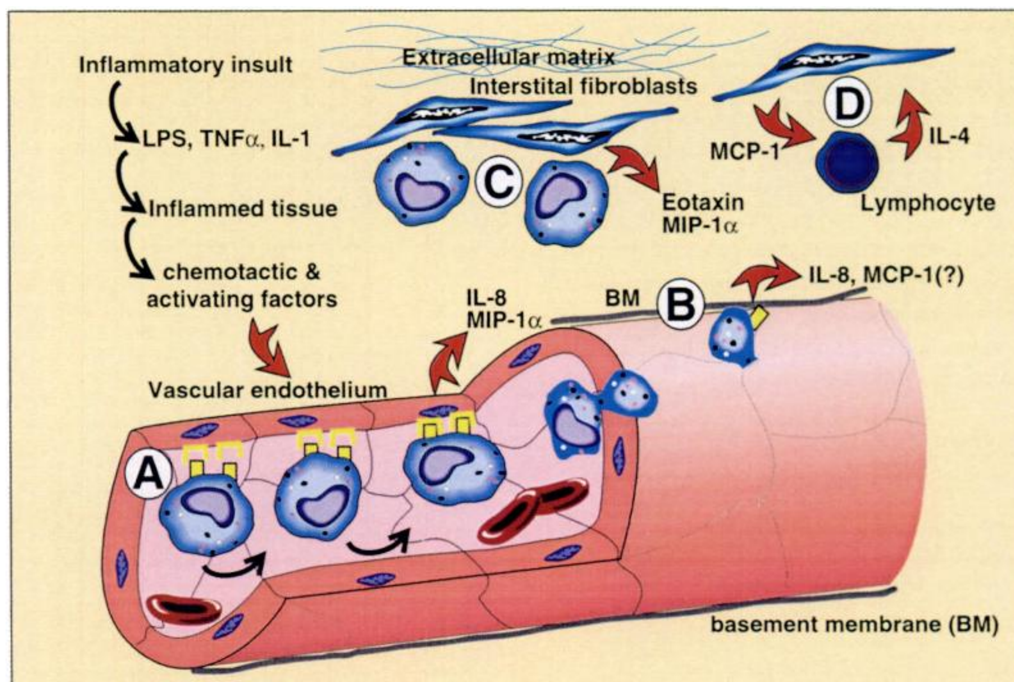

Fig. 2. Adhesion events mediate chemokine expression. After an inflammatory insult, inflamed tissues activate adjacent endothelium. The activated endothelium promotes recruitment of leukocytes such as monocytes. The monocyte-endothelial cell interaction (A), and subsequent monocyte interactions with the basement membrane (B) or interstitial fibroblasts (C) promotes stimulation of chemokine expression. Other types of intimate cell-to-cell interactions, like those between lymphocytes and fibroblasts (D), may also modulate chemokine expression.

cytes [68]. Because different T cell clones have different chemokine receptor profiles, we hypothesize that populations of specific T lymphocytes may be retained or recruited via adhesion-dependent expression of different combinations of chemokines from stromal cells and macrophages found in the chronic lesion (Fig. 2D). Such a process may provide part of the specificity for the generation of a chronic inflammatory process versus resolution of an acute lesion.

Because in vitro studies are incomplete and few in vivo studies have been undertaken, it is difficult to predict the role of adhesion-mediated chemokine expression in human disease. However, anti-CD-18 (β_2 integrin) antibodies significantly attenuated TNF protein expression in a rabbit model of sepsis [69]. In this model, LPS-challenged animals given anti-CD-18 therapy had significantly improved survival rates and other clinical parameters such as blood pressure and blood gas analyses. Because adhesion-mediated cytokine and chemokine expression likely involve similar pathways, this study supports the hypothesis that adhesion-mediated chemokine expression has an important role in vivo. Future experiments will evaluate effects of adhesion molecule-neutralizing antibodies on chemokine expression and pathological endpoints in animal models of human disease. In addition, completion of in vitro studies will hopefully demonstrate the specific receptor-substrate components responsible for chemokine expression in various co-culture models of inflammation. Perhaps most interesting will be extension of fibroblast-lymphocyte co-culture experiments, providing insight for the putative mechanisms of chronic inflammatory disorders. Although fibroblast- or endothelial cell-leukocyte co-cultures were discussed in this review, future investigations will also focus on chemokine expression following leukocyte adhesion to epithelial cells. Other future investigations that warrant close scrutiny involve characterization of chemokine receptor expression, an area lagging far behind that of the receptor ligands. Finally, although stimulation of chemokine expression by soluble mediators is central to the inflammatory process, the adhesion event alone or in combination with exogenous stimuli is probably an important contributor to stimulation of chemokine expression, and thus contributes to pathological outcomes in inflammatory diseases.

ACKNOWLEDGMENTS

This research was supported in part by National Institutes of Health Grants HL-31963, HL-28737, HL35276, P50HL56402, IP50HL46487, and A136302.

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