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Disparate Gene Expression of Chemotactic Cytokines by Human Mononuclear Phagocytes

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Chemotactic cytokines are becoming increasingly recognized as important participants in the coordinate recruitment of specific inflammatory cells. In this manuscript we present data demonstrating that LPS challenged human mononuclear phagocytic cells can express mRNA for neutrophil chemotactic factor/interleukin-8 (NCF/IL-8), but do not express mRNA for monocyte chemotactic protein (MCP). The expression of NCF/IL-8 mRNA was time and dose dependent. This identical stimulus response was also found in peripheral blood neutrophils. These studies demonstrate a disparate production of chemotactic cytokines by macrophages and exemplify the dynamic nature of the chemotactic response.

The recruitment of specific phagocytic cells into an area of inflammation is dependent upon a set sequence of highly regulated events. This sequence includes the coordinate induction of chemotactic factors necessary to elicit the appropriate immune cells during an inflammatory response. A number of unrelated chemotactic mediators previously have been identified which demonstrate redundant mechanisms are involved in cell movement. These factors include C5a, leukotriene B4, bacterial-derived F-met-leu-phe, neutrophil chemotactic factor/interleukin-8 (IL-8) and monocyte chemotactic protein (MCP) (1-5). Interestingly, only the last two chemotactic cytokines have been shown to demonstrate specificity regarding inflammatory cell chemotaxis.

In this presentation, we demonstrate that gene expression for IL-8 and MCP is both stimulus and cell specific. Human peripheral blood monocytes and alveolar macrophages can expresss IL-8 mRNA in response to a number of stimuli, including bacteria-derived lipopolysaccharide (LPS), tumor necrosis factor alpha (TNF), and interleukin-1 (IL-1). Both a

Abbreviations used in this manuscript: NCF/IL-8, neturophil chemotactic factor/interleukin-8; MCP, monocyte chemotactic protein; TNF, tumor necrosis factor-alpha; LPS, lipopolysaccharide; AMO, alveolar macrophage.

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dose and time dependent increase in IL-8 mRNA and bioactivity was observed in response to LPS. In contrast, these mononuclear phagocytic cells did not express MCP mRNA when challenged with the above stimulus. These studies demonstrate that the elicitation of cells to an area of inflammation is a highly regulated event that is controlled, in part, via the specific expression of chemotactic cytokines.

Materials and Methods

Cell Preparations: Fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) was performed in normal nonsmoking volunteers by standard techniques (16). Normal volunteers had no evidence of recent viral or upper respiratory tract infection. The recovered BAL suspension was filtered thorough gauze and centrifuged at 600 x g. The alveolar macrophages (AMO) were resuspended in sterile RPMI-1640 (Whitaker Biomedical Products, Denver, PA) media and washed three times prior to plating. Fifty milliliters of heparinized peripheral blood was obtained by venipuncture and diluted 1:1 with normal saline. Mononuclear cells (PBM) were separated by Ficoll-Hypaque, resuspended in media, and washed three times. Either AMO or PBM were finally resuspended and differential cell counts and viability counts were conducted. The cells were then plated in 100-mm culture plates (Costar, Cambridge, MA) at a concentration of 1 x 10^6 cells/ml.

Reagents: Human recombinant IL-1β with specific activity of 30 U/ng was the generous gift of the Upjohn Company. Human recombinant TNF-α (20 U/ng, specific activity) was a gift of the Cetus Corporation. Lipopolysaccharide (0111:B4) was purchased from Sigma (St. Louis, MO).

mRNA Assessment: Total cellular RNA was extracted using modifications of previously published reports (6,7). Briefly, adherent cells were solubilized in a solution of 25 mM Tris, pH 8.0 containing 4.2 M guanidine isothiocyanate, 0.5% Sarkosyl, and 0.1 M beta-mercaptoethanol. After homogenization, an equal volume of 100 mM Tris, pH 8.0 containing 1.0% SDS and 10 mM EDTA was added and the RNA extracted with chloroform-phenol. The alcohol precipitated RNA was separated by formaldehyde/1% agarose gels and transblotted to nitrocellulose. The baked blots were prehybridized and then hybridized with ^{32}P-5-end-labeled 30-mer oligonucleotide probe. The probes were complementary to either nucleotides 256-285 of published cDNA sequence for human monocyte chemotactic and activity factor/monocyte chemoattractant protein (4,5) or nucleotides 262-291 of a published cDNA sequence for neutrophil chemotactic factor (8). The sequence of the MCP probe was 5'-TTG-GGT-TTG-CTT-GTC-CAG-GTG-GTC-CAT-GGA-3', while the sequence for the NCF probe was 5'-GTT-GGC-GCA-GTG-TGG-TCC-ACT-CTC-ATT-CAC-3'. Blots were stringency washed and autoradiographs were quantitated by laser densitometry. Equivalent amounts of total RNA load per gel lane were assessed by monitoring 28s and 18s rRNA.

Results

Chemotactic Cytokine Gene Expression by Blood Monocytes and Alveolar Macrophages. Initial studies in this set of experiments were designed to assess the ability of stimulated human mononuclear phagocytic cells to express mRNA for specific chemotactic cytokines. Both peripheral blood monocytes and alveolar macrophages were studied for the expression of MCP and IL-8 when challenged with LPS, a potent macrophage stimulus. As shown in Figure 1, Northern blot analysis of total RNA isolated from LPS treated peripheral blood monocytes demonstrated a time dependent induction for IL-8 mRNA expression. Steady state mRNA levels for IL-8 reached a zenith 24 hours post LPS stimulation. On the contrary, steady state levels of

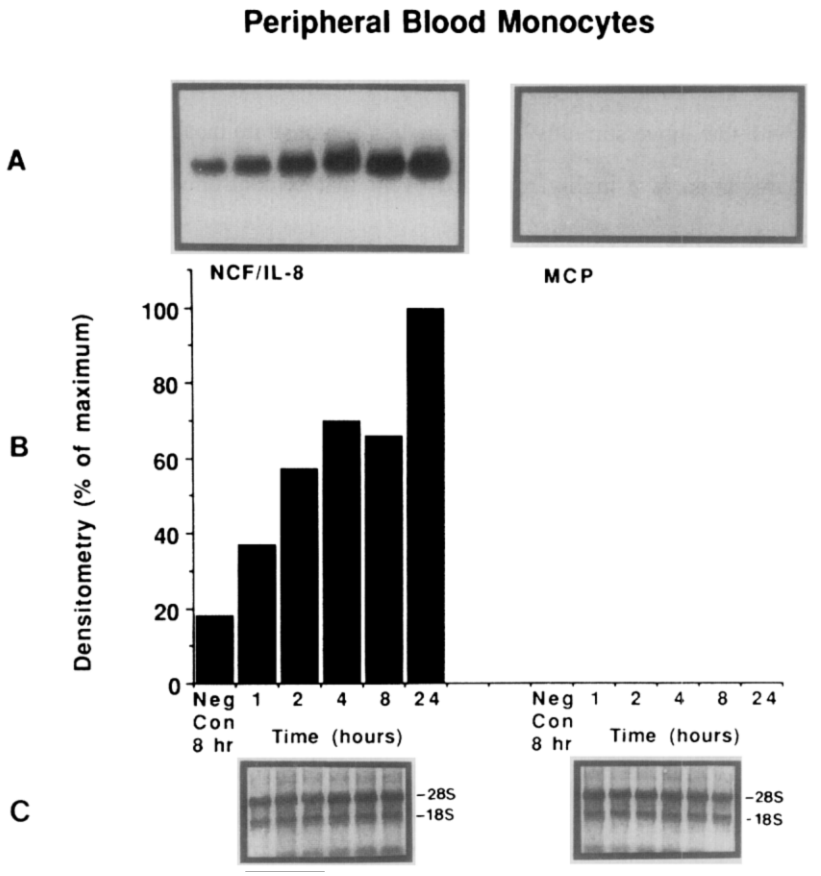

Figure 1. Northern blot analysis demonstrating specific induction of IL-8, but not MCP, mRNA expression by LPS challenged human blood monocytes. Adherent monocytes were stimulated with 1 ug/ml LPS and at the indicated times assessed for the expression of chemotactic cytokines. Similar results were found with either IL-1 or TNF stimulation. A) Northern blot; B) laser densitometry of the Northern blots; and C) 28S and 18S rRNA of the blots in 'A'.

MCP mRNA were undetectable at any time point after LPS treatment (Figure 1). Even 48 hours post the addition of LPS the disparate expression of IL-8 and MCP steady state mRNA could still be observed (data not shown). Human alveolar macrophages challenged with LPS demonstrated a similar expression pattern for IL-8 and MCP steady state mRNA (Figure 2). As with the peripheral blood monocytes, LPS treated alveolar macrophages also expressed IL-8 mRNA in a kinetic fashion with a peak in levels occurring at the 8 rather than 24 hour time point. The expression of MCP mRNA was not evident by LPS treated human alveolar macrophages over a 24 hour period. Although the Northern blot data generated in Figure 2 is based on 1 ug/ml of LPS, no MCP mRNA expression was observed over a four log dose range. Similar results were found when either IL-1 or TNF was used as a stimulus.

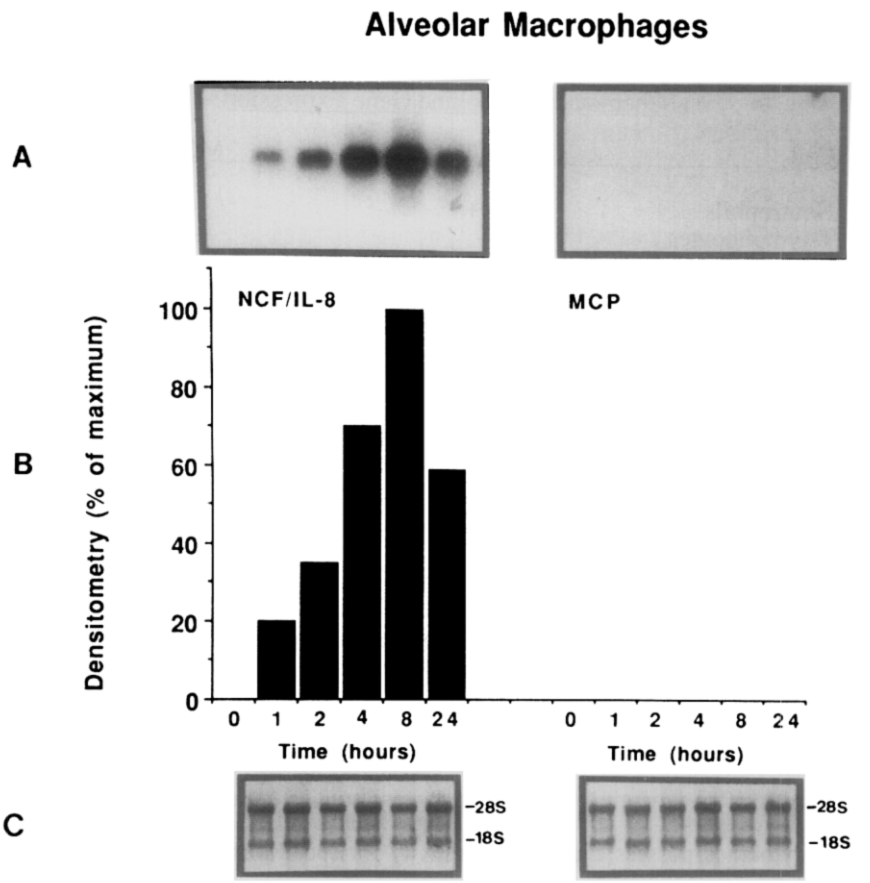

Figure 2. Time dependent induction of IL-8 mRNA expression by LPS treated human alveolar macrophages. As shown by the Northern blot analysis, MCP mRNA was not expressed by the challenged alveolar macrophages, while IL-8 mRNA was significantly induced by 10 ug/ml LPS. Alveolar macrophages stimulated with either IL-1 or TNF demonstrated the same pattern of study state mRNA for NCF/IL-8 and MCP. A) Northern blot; B) laser densitometry of the Northern blots; and C) 28S and 18S rRNA of the blots in 'A'.

Chemotactic Cytokine Gene Expression by Other Inflammatory Cells. In addition to mononuclear phagocytic cells, LPS treated neutrophils and lymphocytes were also studied for IL-8 and MCP mRNA expression. As shown in Table 1, human neutrophils treated with 1 ug/ml LPS expressed abundant IL-8 mRNA but failed to express detectable levels of MCP. The expression of chemotactic cytokine mRNA by neutrophils is identical to that of blood monocytes and alveolar macrophages. In contrast, human B lymphocytes treated with LPS failed to express IL-8 mRNA, but did synthesize MCP mRNA in response to LPS. T-lymphocytes produced neither IL-8 nor MCP mRNA in response to LPS treatment.

Table 1. Inflammatory cell specific induction of NCF/IL-8 and MCP mRNA

	Chemotactic Cytokine Gene Expression	
Cell	NCF/IL-8	MCP
Neutrophils	++++	-
T-lymphocytes	-	-
B-lymphocytes	-	++
Blood monocytes	+++++	-
Alveolar macrophages	+++++	-

Each of the above immune cell populations were challenged with 1 ug/ml LPS and assessed for the expression of specific chemotactic cytokine mRNA.

Discussion

The influx of blood leukocytes into an area of immune reactivity is one of the earliest events that occurs during inflammation. This influx is initially characterized by a predominant neutrophilic infiltrate followed by a monocytic infiltrate. Although a number of mediators have been identified that can recruit immune cells, most of these possess little specificity with regard to the movement of phagocytic blood cells. Recent studies have shown that a novel class of chemotactic cytokines possess chemotactic activity for specific inflammatory cell populations. Interleukin-8 appears to possess specific chemotactic activity mainly for neutrophils (3,8), while MCP is active only for monocytes (4,5).

In addition to influencing the recruitment of only specific inflammatory cell populations, we now show that gene expression for the individual chemotactic cytokines is cell specific. While phagocytic inflammatory cells (such as monocytes, neutrophils, and alveolar macrophages) can express IL-8 mRNA in response to LPS, these same cells are unable to synthesize MCP mRNA. Mononuclear phagocytes synthesize an abundance of neutrophil chemotactic factor, but do not express the above monocyte chemotactic protein. Thus, other cellular participants of the inflammatory response, such as endothelial cells and fibroblasts (9) may be increasingly important in recruiting monocytes to an active lesion. These studies lend support to the dynamic interactions necessary for the coordinate expression of specific chemotactic cytokines.

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