American Journal of Pathology, Vol. 138, No. 6, June 1991

Rapid Communication

Interleukin-4 Induces the Synthesis and Secretion of MCP-1/JE by Human Endothelial Cells

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The authors have demonstrated that human interleukin-4 (IL-4) induces increased expression of the mRNA encoding the monocyte-specific chemoattractant and activator, MCP-1/JE, in human endothelial cells (EC). In addition, treatment of ECs with IL-4 resulted in the synthesis and secretion of MCP-1/JE protein. While IL-4 did not significantly influence the induced expression of MCP-1/JE mRNA by interleukin-1 (IL-1) or tumor necrosis factor, concomitant treatment with IL-4 and IL-1 caused more secretion of MCP-1/JE protein than either cytokine alone. These results suggest that EC-produced MCP-1/JE may mediate some of IL-4's effects on monocyte physiology in vivo, ***including IL-4's anti-tumor properties. (Am J Pathol 1991, 138:1315–1319)***

Interleukin-4 (IL-4) is a T-helper lymphocyte-produced cytokine that displays pleiotropic effects *in vitro*. Initial studies described its ability to stimulate murine B-lymphocyte proliferation,[1] class switching,[2] and increased class II major histocompatibility (MHC) expression.[3,4] Interleukin-4 was later shown to act on human B cells to promote isotype switching and to increase expression of low-affinity Fc ε receptors (CD23).[5] Human IL-4 also can stimulate T-lymphocyte proliferation[6] and influence the generation of cytolytic T cells.[7] In addition, there is substantial evidence that IL-4 is involved in regulating monocyte and macrophage function. For example, murine IL-4 can activate the tumoricidal properties of mouse peritoneal macrophages *in vitro*,[8] while human IL-4 inhibits the respiratory burst[9,10] and IL-1,[11,12] tumor necrosis factor (TNF),[12] IL-6,[13,14] and IL-8[15] secretion by human monocytes. Interleukin-4 may contribute to mononuclear leukocyte recruitment into tissues by inducing vascular endothelial cell (EC) expression of vascular cell adhesion molecule-1 (VCAM-1), an adhesive molecule for monocytes and lymphocytes.[16–18]

Much less is known about IL-4's physiologic function *in vivo*. Recently it has been found that IL-4 can activate murine macrophages to prevent tumor growth.[19] However IL-4 does not display chemotactic activity for monocytes *in vitro*, and it is not known whether IL-4 acts directly on monocytes or induces the expression of an intermediate monocyte-specific cytokine. A candidate mediator would be monocyte chemoattractant protein-1 (MCP-1), a monocyte-specific chemoattractant[20,21] and stimulant[22,36] that is the product of the JE gene.[23–25] We previously demonstrated that IL-1–, TNF-, or IFN-γ–activated human endothelial cells secrete MCP-1/JE,[26] and recently showed that MCP-1/JE activates monocyte tumoricidal activity *in vivo*.[27] Here we report that IL-4 induces ECs to synthesize and secrete MCP-1/JE. We propose that this action of IL-4 may contribute to the appearance of activated monocytes at the site of IL-4 production.

Materials and Methods

Cell Culture and Cytokines

Human ECs were isolated from three to five umbilical veins by collagenase digestion, pooled, and serially subcultured. Culture conditions and reagents have been described elsewhere.[28] Cytokines used in these studies were added to ECs in complete medium and included recombinant human IL-4 (expressed in yeast, 1.0×10^8

Supported by NIH grants CA53091 awarded to B. J. Rollins and HL36003 awarded to J. S. Pober.

Accepted for publication April 10, 1991.

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U/mg, Genzyme Corp., Boston, MA), IL-1β (expressed in *Escherichia coli,* 1.0×10^7 U/mg, a gift of Dr. A. Shaw, Biogen, Geneva, Switzerland), and human TNF (expressed in *E. coli,* 2.5×10^7 U/mg, a gift of Biogen, Cambridge, MA). The IL-4 preparation contained less than 3 ng/ml endotoxin. All experiments were conducted at subcultures three to five, by which time mononuclear phagocytes were not detectable in these cultures.

RNA Analysis

RNA was isolated from 3–5 × 10^6 cells by guanidinium isothiocyanate extraction followed by centrifugation through cesium chloride.[29] RNA was electrophoretically fractionated through a 1.5% agarose-2.2 mol/l (molar) formaldehyde gel and transferred to nitrocellulose or nylon-based filters. Baked or ultraviolet-cross-linked filters were hybridized at 42°C as described.[24] Probes were labeled by nick translation or random priming to a specific activity of 18^8–10^9 CPM/μg and included the following: MCP-1/JE, the XhoI fragment from phJE-34;[23] and NAP-1/IL-8, a PstI-EcoRI fragment from the NAP-1/IL-8 cDNA cloned into pKK233-2, a gift of Dr. S. L. Kunkel (University of Michigan Medical School, Ann Arbor, MI). Densitometry measurements were performed using an LKB Ultrascan enhanced laser densitometer (Pharmacia LKB Biotechnology Inc., Piscataway, NJ).

Immune Precipitation

Four 10-cm tissue culture dishes, each containing 2 × 10^6 confluent endothelial cells in growth medium, were treated with 10 U/ml IL-1β, 300 U/ml IL-4, IL-1β and IL-4 at the same concentrations, or nothing. After 4 hours, [^{35}S]-methionine (DuPont NEN, Boston, MA) was added to 333 mCi/ml. At the indicated times, 0.5 ml medium was withdrawn from each flask and made 2 mmol/l (millimolar) in phenylmethylsulfonyl fluoride (PMSF). Cells and debris were removed by centrifugation, an equal volume of RIPA buffer (50 mmol/l TRIS-HCl [pH 7.5], 150 mmol/l NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 0.5% deoxycholate, 1 mmol/l PMSF) was added, and immune precipitation carried out with anti-JE antiserum or control serum as described[23] after preclearing with control serum and *Staphylococcal* protein A beads (BioRad, Richmond, CA).

Results

IL-4 Induces MCP-1/JE mRNA Expression in Endothelial Cells

As noted previously by us[26] and by others,[30–32] human ECs express low levels of MCP-1/JE mRNA under standard culture conditions (Figure 1). Treatment of human ECs with 10 to 1000 U/ml IL-4 for 24 hours induces increased expression of MCP-1/JE mRNA in a dose-dependent fashion (Figure 1). Treatment of these cells with 100 U/ml IL-4 for increasing periods of time leads to increasing levels of MCP-1/JE mRNA expression up to 72 hours. MCP-1/JE expression was unlikely to be due to endotoxin contamination for several reasons. First ECs do not respond to less than 100 ng/ml endotoxin, while the maximum endotoxin level of the IL-4 preparation was 3 ng/ml. Second, while endotoxin induces IL-8 expression in ECs,[33] IL-4 did not (Figure 2). Third boiling destroyed IL-4's ability to induce MCP-1/JE expression (data not shown).

IL-4 Does Not Affect IL-1– or TNF-induced MCP-1/JE Expression

Interleukin-4 has been reported to modulate the effects of other cytokines on various target cells. For example, IL-4 inhibits IL-1– or TNF-induced secretion of IL-8 by monocytes.[15] Figure 2 shows that concomitant treatment with 300 U/ml IL-4 does not alter the IL-1– or TNF-induced expression of MCP-1/JE mRNA in ECs. We also confirm that IL-4 neither induces IL-8 mRNA expression in ECs nor prevents the induction of IL-8 mRNA expression by IL-1 or TNF[34] (Figure 2).

IL-4 Stimulates Endothelial Cells to Synthesize and Secrete MCP-1/JE

Having demonstrated MCP-1/JE mRNA induction by IL-4, we wanted to determine whether IL-4 induced ECs to

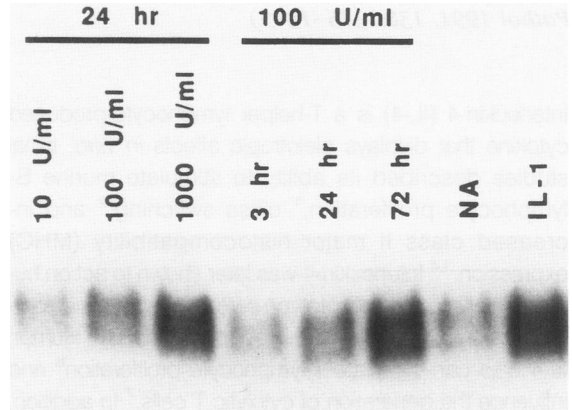

Figure 1. *Human ECs were treated as indicated, varying the amount or time of exposure to IL-4. Il-1β–treated ECs (10 U/ml for 24 hours) is included as a positive control, and NA (no addition) indicates untreated ECs in unchanged growth medium for 72 hours. RNA was isolated and MCP-1/JE mRNA content was analyzed by Northern blotting as described in Materials and Methods. Similar levels of MCP-1/JE mRNA induction by IL-4 were observed in five additional independent experiments.*

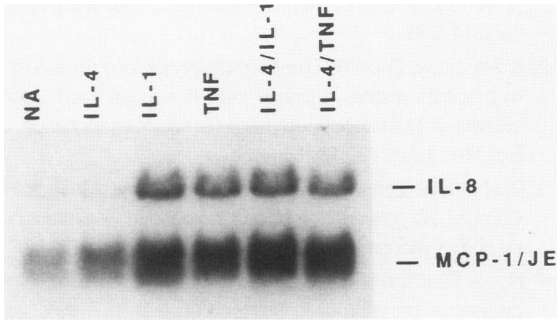

Figure 2. *Human ECs were treated with IL-4 (300 U/ml), IL-1β (10 U/ml), and TNF (100 U/ml) alone or in combination as indicated. NA indicates no cytokine treatment. Levels of MCP-1/JE mRNA and IL-8 mRNA were determined on the same Northern blot as described in Materials and Methods. Similar results were obtained in two additional independent experiments.*

synthesize and secrete MCP-1/JE protein. Figure 3 shows that after 24 hours of IL-4 treatment, ECs secrete little detectable MCP-1/JE protein. As reported previously,[26] treatment with IL-1 during the same period of time results in MCP-1/JE secretion. By 72 hours, however, IL-4 induces MCP-1/JE synthesis and secretion to the same level as 72 hours of IL-1 treatment. Although we did not observe an interaction between IL-4 and IL-1 at the mRNA level, concomitant treatment with IL-4 and IL-1 appears to enhance the synthesis and secretion of MCP-1/JE protein compared to either cytokine alone.

Discussion

We have demonstrated that treating human endothelial cells with IL-4 induced (1) the accumulation of MCP-1/JE mRNA and (2) the synthesis and secretion of MCP-1/JE protein. In an earlier communication, we demonstrated that MCP-1/JE secreted by these cells was active as a monocyte-specific chemoattractant and that this protein accounted for nearly all the monocyte chemoattractant activity secreted by IL-1–treated endothelial cells.[26] In most natural and recombinant expression systems, human MCP-1/JE is synthesized as two proteins of M_r 15,000 (α form) and 11,000 (β form), differing in their extent of O-linked glycosylation.[35] Like IL-1, IL-4 induced endothelial cells to secrete almost exclusively the α form.

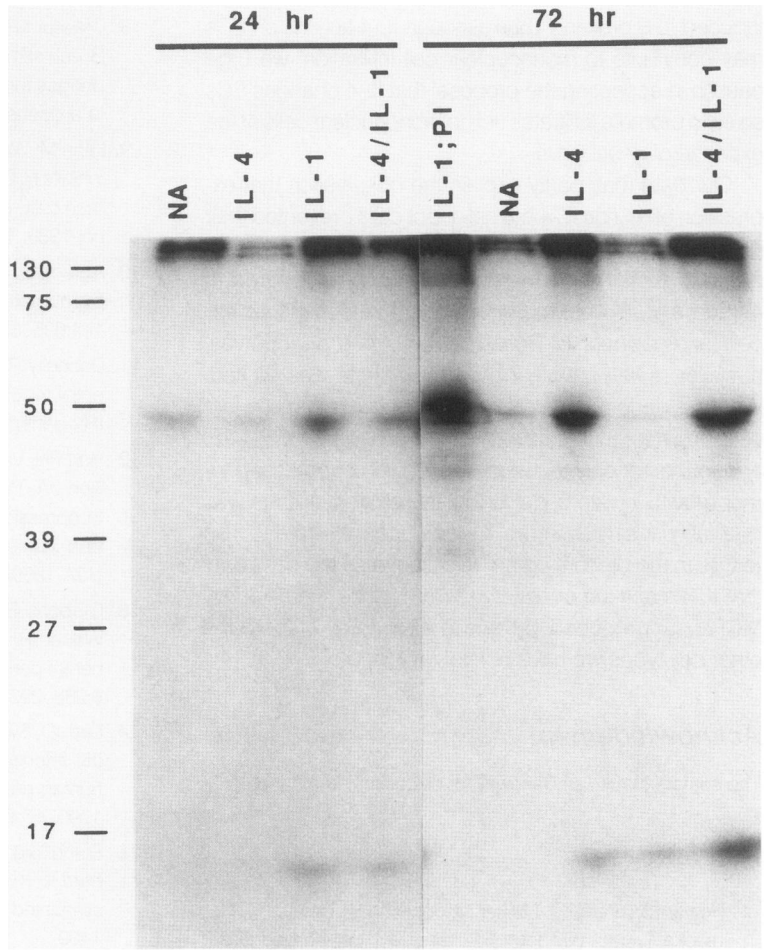

Figure 3. *Human ECs were treated with Il-4 (300 U/ml), IL-1β (10 U/ml), both cytokines in combination, or no addition (NA). After 4 hours, [^{35}S]methionine was added as described in Materials and Methods. After a further 24-hour and 72-hour incubation, aliquots of medium were removed and analyzed by immune precipitation using anti–MCP-1/JE antiserum or preimmune serum (PI). Molecular size markers in kilodaltons are indicated. Specifically precipitated MCP-1/JE-α protein of M_r 15,000 is observed in the lanes from cytokine-treated cells. IL-4 induction of MCP-1/JE protein was confirmed in an additional independent experiment.*

Endothelial cells remain one of the few cell types to display differential synthesis of the two forms of human MCP-1/JE.

Our results help to extend the connection between IL-4 and the physiology of mononuclear phagocytes. Although IL-4 itself is not a chemoattractant for monocytes, local release of this cytokine could stimulate endothelial cells to secrete MCP-1/JE. Monocytes that adhere to the vascular wall by means of IL-4–induced VCAM-1, displayed on the same endothelial cells,[16–18] may be uniquely positioned to respond to EC-produced MCP-1/JE. In the milieu of a general inflammatory response, IL-4's interactions with other cytokines may result in a complex set of signals. For example, IL-4 can inhibit the secretion of IL-8 by activated monocytes, suggesting that IL-4 can down regulate a neutrophil response.[15] Interleukin-4 has also been reported to inhibit IL-1–induced expression of ICAM-1 and ELAM-1.[17] However we saw no effect of IL-4 on the expression of IL-1– or TNF-induced IL-8 mRNA in ECs (Figure 2), and when normalized to an irrelevant surface antigen, we have not been able to confirm an effect of IL-4 on IL-1–induced ELAM-1 or ICAM-1 expression by ECs (data not shown). (We have not examined the effect of IL-4 on the secretion of IL-1– or TNF-induced IL-8 protein.) Thus although IL-4 actions on ECs may contribute to mononuclear cell infiltration, we urge caution in accepting the proposal that IL-4 changes ECs so as to promote infiltrates rich in mononuclear cells at the expense of neutrophils.

Our data may partly explain the observation that expression of murine IL-4 by malignant cells prevented their growth *in vivo* after subcutaneous injection.[19] The mechanism of IL-4–induced tumor suppression appeared to rely on a local accumulation of activated mononuclear cells and eosinophils. Based on IL-4's *in vitro* properties, however, it was not clear how this infiltrate was elicited. We have performed similar experiments using malignant cells engineered to express human or murine MCP-1/JE, and found not only that such expression suppressed tumor growth *in vivo* but also that the elicited infiltrate was qualitatively similar to the IL-4–induced infiltrate.[27] The results in the present communication raise the possibility that IL-4's anti-tumor effect *in vivo* may be mediated by MCP-1/JE produced by endothelial cells, or perhaps other cell types we have not yet identified.

Acknowledgment

The authors thank Teri Bakewell for cell culture assistance.

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