CONCISE REPORT

Tumor Necrosis Factor (TNF)-alpha but not TNF-beta Induces Secretion of Colony Stimulating Factor for Macrophages (CSF-1) by Human Monocytes

By W. Oster, A. Lindemann, S. Horn, R. Mertelsmann, and F. Herrmann

Tumor necrosis factor (TNF)-alpha has been identified as a major inducer of colony stimulating factor (CSF)-secretion by human vascular endothelial cells and fibroblasts. In the present study we assessed the capacity of TNFs to induce release of CSF-1 from highly purified peripheral blood monocyte preparations. Whereas monocytes do not accumulate CSF-1 messenger (mRNA) constitutively and consequently do not produce CSF-1 protein, CSF-1 mRNA and protein secretion became detectable, when monocytes were cultured in the presence of TNF-alpha, that was synergistically enhanced by interferon-gamma (IFN-gamma). However, under identical experimental conditions TNF-beta failed to induce monocyte CSF-1 synthesis. Cultures of monocytes in the presence of TNF-beta before addition of TNF-alpha abolished the CSF-1 inducing capacity of TNF-alpha, suggesting that TNF-beta may act as an antagonist to TNF-alpha for CSF-1 production. These data point out a previously unrecognized function of TNF-alpha to modulate CSF-1 release by monocytes and demonstrate disparate biological properties of different TNF species in hematopoiesis.

MATERIALS AND METHODS

Preparation of monocytes and T cells. Peripheral blood derived mononuclear cells were isolated from leukapheresis residues of consenting healthy volunteer donors by Ficoll-Hypaque gradient (density 1.078 g/dL) centrifugation. T cells were recovered with AET (2-aminoethylisothiouronium bromide hydrobromide)-treated sheep RBCs (SRBCs) (5% vol/vol solution). Monocytes were separated by two sequential adherence steps of the SRBC-rosette negative fraction. Individual cell fractions, assessed by morphology (Wright Giemsa and alpha-naphthyl acetate esterase [ANAE] staining) and immunofluorescence analysis, using monoclonal antibodies (MoAb) to the T11, Mo2, and Bi antigens, revealed preparations of >98% purity. Monocytes or T cells were plated at 10^6 cells/mL in RPMI 1640 supplemented with 5% fetal calf serum (FCS), penicillin/streptomycin, L-glutamine and sodium pyruvate in plastic petri dishes (35 mm/well; Corning, New York). Recombinant human (rh) TNF-alpha or TNF-beta (kindly provided by Genentech, San Francisco, through R. Flener, Boehringer, Vienna) was added to cultures at 50 ng/mL (specific activity of rhTNF-alpha or rhTNF-beta of 5 x 10^8 U/mg, respectively). In selected experiments cultures received IFN-gamma (0.05 ng/mL; specific activity of IFN-gamma 4 x 10^8 U/mg; kindly provided by Genentech). In control cultures the addition of TNF-alpha, TNF-beta, and IFN-gamma was omitted. Cell-free supernatants were collected after 18 to 72 hours of culture. To neutralize the effects of carry-over of TNF preparations on growth of hematopoietic progenitor cells, TNF-monoocyte conditioned media (TNF-MCM) and TNF-T cell conditioned media (TNF-TCM) were preincubated with MoAb to TNF-alpha or TNF-beta (kindly provided by Genentech) at concentrations sufficient to neutralize threefold more TNF-alpha or TNF-beta than was originally added. For RNA analysis cultures were performed for six to 24 hours or in selected experiments for up to 96 hours.

Northern blot analysis. Total RNA was prepared using the guanidium/cesium chloride method and denatured with glyoxal and dimethylsulfoxide as described previously after quantification by spectrophotometry. Aliquots of each RNA were stained in minigels.
before denaturation with ethidium bromide to visualize 28 S and 18 S ribosomal RNA bands, in order to confirm quantification and integrity of the RNA samples. Identical amounts of total RNA (10 μg) were size-fractionated in 1.2% agarose gel electrophoresis, transferred to nylon base paper by capillary blot using 10 × SSC and baked for two hours at 18°C.21 Specific c-DNA probe for M-CSF (1 kb Bgl I-PST I fragment cloned in PUC 18, kindly provided by P. Ralph, Cetus Corporation, Emeryville, CA) was radiolabeled as reported previously22 with a specific activity of 2.5 to 3.5 × 10^6 cpm per μg DNA. Hybridization was performed in 50% formamide, 1% SDS, 5 × Denhardt’s, 6 × SSPPE at 42°C for 18 hours. Filters, washed with 2 × SSC at 27°C, 0.1 × SSC, 1% SDS at 65°C and 0.1 × SSC at 27°C, were exposed to Kodak XAR5 with Dupont Cronex intensifying screens.

Murine CFU-GM colony assay. CFU-GM were assayed in a double layer agar culture system as previously described.23 Underlayer (0.5 mL) were composed of 0.5% agar (Agar Noble, Difco, Detroit, IL) in Iscove’s modified Dulbecco’s minimum essential medium (IMDM) supplemented with L-glutamine, penicillin/streptomycin, 20% FCS and 5 × 10^-3 mol/L 2-mercaptoethanol. As a potential source of CSF, media conditioned by monocytes (MCM) cultured for 48 to 72 hours in the presence or absence of TNF-alpha (50 ng/mL) and TNF-beta (50 ng/mL), respectively were added to the underlayers at 10% vol/vol final concentration. Overlayers (0.5 mL) were composed of 0.3% agar in the same medium and contained 5 × 10^5/mL murine (BDF, 1 mice) bone marrow cells (obtained from femures). CFU-GM were enumerated at day 7 and lineages of colonies were assessed by cytochemical in situ staining of whole agar cultures.23 Naphthol-AS-D-chloroacetate esterase staining (CAE) detects granulocytic colonies, ANAE staining macrophage colonies.

RESULTS AND DISCUSSION

We have previously shown, that monocytes are a potential source of CSF-1 when induced with phorbol esters.9 In the present study we investigated effects of TNF-alpha and TNF-beta on CSF-1 secretion by monocytes. Whereas monocytes do not transcribe CSF-1-gene constitutively (Fig 1, lane a) CSF-1 mRNA of 4.5 kb length became detectable, when monocytes were cultured in the presence of TNF-alpha for six hours (Fig 1, lane b) to 24 hours (not shown). In contrast, under identical conditions, TNF-beta failed to induce CSF-1 mRNA synthesis by monocytes (Fig 1, lane c); concentrations of TNF-beta >50 ng/mL (up to 500 ng/mL) and prolongation of incubation periods to >24 hours (up to 96 hours) did not affect CSF-1 processing (not shown). Autoradiograms exposed for as long as 14 days continued to show no signal for CSF-1 mRNA, when monocytes were incubated in the presence of TNF-beta. As in all cultures systems like the one used here, it is difficult to prove that the CSF-1 activity is actually released by monocytes and not by contaminating T cells. To determine if the effect of TNF-alpha on induction of CSF-1 release was on monocytes and not on possibly contaminating T cells, experiments were performed, in which the effects of TNFs on expression and secretion of CSF-1 by resting T cells were investigated.

Under experimental conditions that resulted in CSF-1 release by monocytes, T cells were unable to express or release CSF-1 in response to TNF-alpha (Fig 1, lane e). Since TNF-alpha and TNF-beta compete for binding to a common receptor domain we investigated the possibility, whether TNF-beta acts as an antagonist to TNF-alpha for CSF-1 production by culturing monocytes sequentially in the presence of both factors. Treatment of monocytes with TNF-beta for six hours before addition of TNF-alpha abolished the potential of TNF-alpha to induce CSF-1 mRNA accumulation (Fig 2).

Since IFN-gamma synergizes with TNFs in a variety of systems,14,16 effects of IFN-gamma on TNF-alpha inducible CSF-1 mRNA accumulation was investigated. To this end, RNA of monocytes was probed with a CSF-1 c-DNA that
**Table 1. CSF-1 Secretion by Human Monocytes**

<table>
<thead>
<tr>
<th>Source</th>
<th>Monocyte (ANAE +)</th>
<th>Granulocyte (CAE +)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>11 ± 3†</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>MCM</td>
<td>16 ± 2</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>TNF-alpha MCM</td>
<td>116 ± 3</td>
<td>120 ± 2</td>
</tr>
<tr>
<td>TNF-beta MCM</td>
<td>18 ± 3</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>TCM</td>
<td>12 ± 3</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>TNF-alpha TCM</td>
<td>14 ± 4</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>TNF-beta TCM</td>
<td>13 ± 2</td>
<td>12 ± 2</td>
</tr>
</tbody>
</table>

Abbreviation: Exp, experiment.

* Purified cell populations (monocytes or T cells) were cultured at 1 x 10⁶/mL for 48 to 72 hours in the presence or absence of TNF-alpha or TNF-beta (50 ng/mL). Cell-free media were used as potential stimuli for murine CFU-GM at final concentrations of 10% vol/vol: Monocyte conditioned medium (MCM), in the presence of TNF-alpha (TNF-alpha MCM) or TNF-beta (TNF-beta MCM), T-cell conditioned medium (TCM) in the presence of TNF-alpha (TNF-alpha TCM) or TNF-beta (TNF-beta TCM).

†Colony numbers are means of triplicate CFU-GM assays (±SD).

**References**


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