Brief Definitive Report

γ INTERFERON ENHANCES MACROPHAGE TRANSCRIPTION OF THE TUMOR NECROSIS FACTOR/CACHECTIN, INTERLEUKIN 1, AND UROKINASE GENES, WHICH ARE CONTROLLED BY SHORT-LIVED REPRESSORS

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Immune interferon (IFN-γ) released by stimulated T lymphocytes is believed to be the major macrophage activating factor (MAF) (1). IFN-γ affects the genetic program of macrophages, and increases the expression of major histocompatibility complex class II antigens and of the immunoglobulin Fc receptor (1). Depending upon their state of activation, macrophages release a number of proteins that are thought to play an important role in inflammation, such as interleukin 1 (IL-1) (1) and the urokinase-type plasminogen activator (u-PA) (2). Tumor necrosis factor/cachectin (TNF-α) is also a macrophage product, and it is released in large amounts upon stimulation by bacterial endotoxin (LPS). TNF-α has a broad range of biological activities, including a cytotoxic effect against certain tumor cells and participation in the destruction of parasites (3).

To further explore the mode of action of IFN-γ on macrophages, we chose to study its effect on the expression of the genes coding for these secreted proteins. We show here that IFN-γ enhances transcription of the TNF-α, IL-1, and u-PA genes in mouse peritoneal macrophages, resulting in a large increase in their mRNA levels. While investigating whether these effects of IFN-γ require the synthesis of new proteins, we observed that the inhibition of protein synthesis by cycloheximide rapidly induces the transcription of these three genes, which thus appears to be under the control of short-lived repressors.

Materials and Methods

Macrophage Culture. Peritoneal exudate cells were recovered from 3–5-mo-old AKR, CBA/J, B10A, or C3H/HeJ mice 4 d after a single intraperitoneal injection of 1 ml of aged thioglycollate broth (2.9 g/ml; Difco, Detroit, MI), and incubated for 10–18 h in DMEM (Gibco Laboratories, Grand Island, NY) supplemented with penicillin, streptomycin, pyruvate, and 5% heat-inactivated (56°C, 30 min) FCS; the adherent macrophages population (2) was then washed with PBS and reincubated with supplemented DMEM for at least 1 h before start of the experiments. Adherent resident peritoneal cells were prepared in a similar way.

Isolation and Analysis of Macrophage RNA. Isolation of total cellular RNA and hybrid-

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izations with ^{32}P-labelled cRNA probes have been described (4). The 652 bp Pst I–Hind III fragment of mouse u-PA cDNA clone pDB15 (5) (from position 427 to 1,078) was subcloned into pSP64 (6); the 1,750 bp Eco RI–Pst I fragment of mouse TNFα cDNA clone pAT153-trp-MTNF85 (kind gift from Bernard Allet, Biogen, Geneva, Switzerland), containing the 696 bp Taq I–Eco RI fragment of p-mTNF (7) (from position 418 to 1,114) as well as the 416 bp Pst I–Pvu II fragment of mouse IL-1 cDNA clone pmIL1 (S. Kossodo, unpublished data) (from position 362 to 778 [8]) were subcloned into pSP65 (6).

Run-on Transcriptional Analysis. Nuclei from adherent peritoneal cells ($0.3–1.0 \times 10^7$) were prepared as described (9). After in vitro elongation (10), the RNA was extracted essentially as described (9); instead of TCA precipitation, ethanol precipitation in 2.5 M ammonium acetate, and Sephadex G-50 chromatography were performed. After ethanol precipitation, the nucleic acids were resuspended at $2–4.5 \times 10^6$ cpm/ml and hybridized to linearized plasmid DNAs (2 μg/dot) immobilized onto nitrocellulose (11). In each experiment, all filters were hybridized with the same amount of ^{32}P-labelled RNA. The plasmids used were those described above, as well as pSP65-v-*fos* (containing the 688 bp Pst I–Sal I fragment of v-*fos*) (12), pSP65-c-*myc* (containing the 1.6 kbp Cla I–Eco RI fragment of v-LMC-41) (13), pSP65-v-*myb* (containing the 0.6 kbp Eco RI–Sac I fragment of v-*myb*) (14), pSP65 and pBR322. After hybridization, the filters were washed as described (11) and exposed at −80°C to Kodak XAR-5 films between Dupont Cronex Parspeed intensifying screens.

Results

Thioglycollate-elicited (TG) mouse peritoneal macrophages from all strains studied, including the LPS-resistant C3H/HeJ strain, have detectable levels of TNF-α, IL-1, and u-PA mRNAs. These levels were increased within 2–4 h after addition of IFN-γ; the magnitude of this induction was approximately fourfold for u-PA and IL-1 mRNAs, and eightfold for TNF-α mRNA (not shown). We analyzed this effect of IFN-γ in nuclear run-on experiments, which assay the level of gene transcription at the time of nuclei isolation. Although the relative transcriptional levels of the three genes in untreated cells varied somewhat between different experiments, the highest transcription was consistently that of TNF-α, and the lowest that of IL-1. After 3 h of exposure to IFN-γ, enhanced levels of transcription were observed for all three genes (Fig. 1 *A*). Similar results were obtained with TG-macrophages from C3H/HeJ mice, and with cultures performed in the presence of polymyxin B, which binds and inactivates LPS (not shown).

In run-on experiments performed with resident (R) macrophages, IFN-γ also enhanced transcription of the TNF-α, IL-1, and u-PA genes (Fig. 1 *B*). The basal levels of transcription of these three genes were lower in R-macrophages than in TG-macrophages; this was particularly evident for the u-PA gene. Activation of R-macrophages with IFN-γ resulted in transcriptional levels comparable to (TNF-α and IL-1) or approaching (u-PA) those in unstimulated TG-macrophages. Similarly, in cultures containing polymyxin B, transcription of the three genes was also observed, and it was enhanced by IFN-γ (not shown). IFN-γ did not trigger a general increase in macrophage transcriptional activity, since the amount of ^{32}P-labelled RNA synthesized by stimulated and control nuclei were comparable; in addition, transcription of the protooncogene c-*fos* was decreased, and that of c-*myc* and c-*myb*, two protooncogenes which, like c-*fos*, code for nuclear proteins, were unaffected in IFN-γ-treated cells.

To examine whether the enhancement of transcription by IFN-γ is mediated by newly synthesized proteins, we performed experiments in the presence of

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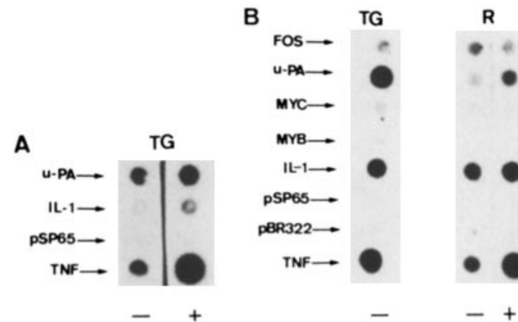

FIGURE 1. Stimulation of TNF-α, u-PA, and IL-1 transcription by IFN-γ in mouse macrophages (*A* and *B*), and comparison of their transcription in R- versus TG-macrophages (B). Nuclei were prepared from TG- and R-macrophages incubated 3 h with (+) and without (−) IFN-γ (100 U/ml; Genentech, South San Francisco, CA). Labelled RNAs synthesized in run-on transcription assays were hybridized to the indicated plasmid DNAs immobilized on nitrocellulose; the pSP65 and pBR322 plasmid vector DNAs were used to appraise background hybridization.

cycloheximide. Unexpectedly, incubation of TG-macrophages with cycloheximide alone resulted in a massive increase in TNF-α, IL-1, and, to a lesser extent, u-PA mRNA levels (Fig. 2*A*). Similar results were obtained with emetine, another inhibitor of protein synthesis (not shown). Nuclear run-on experiments were carried out to explore whether the effect of cycloheximide was due to mRNA stabilization and/or to increased transcription. Incubation of TG-macrophages with cycloheximide led to increased transcription of the TNF-α, IL-1, and u-PA genes, detectable within 10 min, and maximal at 40–60 min (Fig. 2*B*). The maximal increase was 8-fold for u-PA transcription, and about 50-fold for that of TNF-α and IL-1. Similar results were obtained with R-macrophages (not shown). Cycloheximide treatment did not affect the transcription of several other genes also expressed by TG-macrophages, such as the protooncogenes *c-sis*, *c-myb*, *c-myc*, and *c-fms* (not shown). In contrast, the transcription of the *c-fos* protooncogene was enhanced with kinetics similar to those described here; a possible involvement of the *c-fos* gene in the modulation of macrophage activity will be reported elsewhere (Collart, M. A., D. Belin, J.-D. Vassalli, and P. Vassalli, manuscript submitted for publication).

Discussion

The results presented here show that IFN-γ, which may be the only or main lymphokine from activated T lymphocytes that is responsible for macrophage activation (MAF activity), increases the TNF-α, IL-1, and u-PA mRNA levels in macrophages by enhancing the transcription of these genes. Although the synthesis of the corresponding proteins has not been tested in our experiments, IFN-γ has been previously shown to stimulate the secretion of IL-1 (1) and u-PA (15) by macrophages; these stimulations can now be accounted for by the effect of IFN-γ on u-PA and IL-1 gene transcription. The enhancement of TNF-α gene transcription by IFN-γ suggests that this lymphokine may be the main physiological inducer of TNF-α release by macrophages. In vivo, this effect is probably further amplified, since IFN-γ also increases the number of TNF receptors on TNF-α responder cells (16). It is of interest to note that, although

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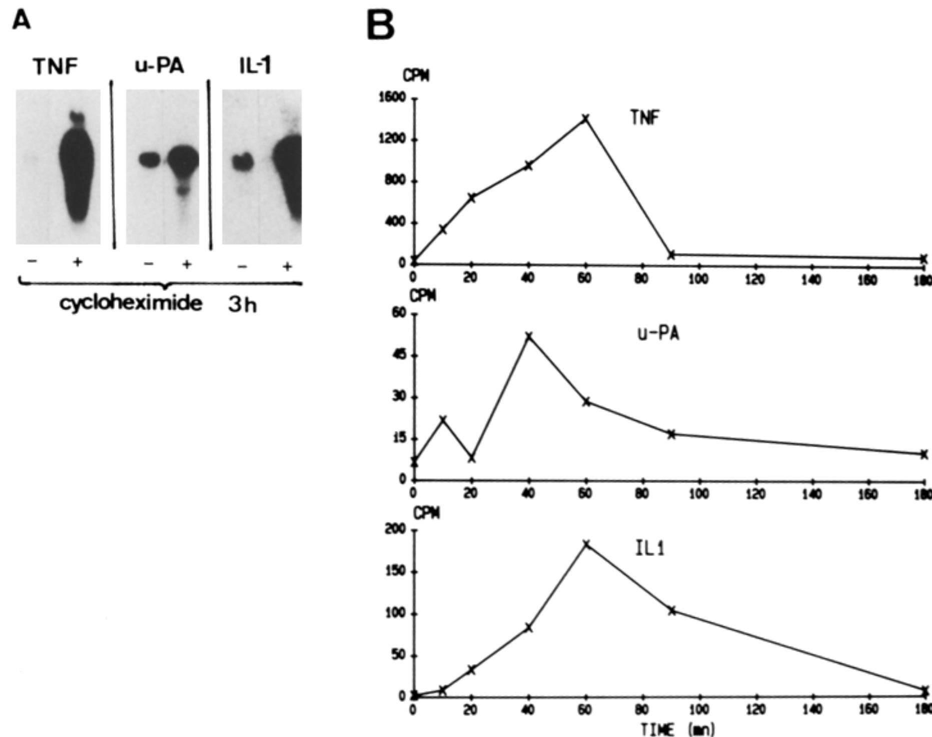

FIGURE 2. Cycloheximide increases TNF-α, u-PA, and IL-1 mRNA levels (*A*) and transcription (*B*) in TG-macrophages. (*A*) Total RNA was extracted from TG-macrophages incubated 3 h with (+) or without (−) cycloheximide (10 μg/ml, Sigma Chemical Co., St. Louis, MO), and analyzed by northern blot hybridization (10 μg/lane) with cRNA probes specific for TNF-α, u-PA, and IL-1. Different exposure times were used for the three filters. (*B*) Nuclei were prepared from TG-macrophages incubated for the indicated times with cycloheximide (10 μg/ml), and run-on transcriptions were performed. After hybridization of the labelled RNAs, spots were cut and counted for 30 min by Cerenkov radiation. Background hybridization to plasmid vector DNA was substracted.

both LPS (17) and IFN-γ enhance the transcription of the TNF-α gene in macrophages, their mode of action appears to be otherwise quite distinct, because LPS decreases the transcription of the u-PA gene and increases that of the *c-fos* gene (Collart et al., manuscript submitted for publication), whereas IFN-γ has the opposite effects.

The very rapid and marked enhancement of transcription of the TNF-α, u-PA, and IL-1 genes observed after the arrest of protein synthesis was unexpected. Transcription induction by cycloheximide has already been reported in a few cases (18). The likeliest explanation is that one or several short-lived repressors normally prevent or decrease the transcription of these genes. Alternative explanations have been considered, including the production of hypothetical nonprotein regulators.

The three genes studied here code for secreted products involved in the inflammatory reaction, and they are transcribed both in resting and inflammatory macrophages, although at different rates. It is thus noteworthy that the transcription of these genes is apparently kept under negative control by repressor protein(s), a situation that may faciliate the rapid modulation of their expression.

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The same observation applies to the protooncogene c-*fos*, whose transcription responds to the modulation of macrophage functional activity (Collart et al., manuscript submitted for publication). In contrast, the transcription of other genes, such as the protoocogenes *fms* and *sis* (19, 20), is apparently not subjected to the same regulatory mechanisms, since it was not increased in cycloheximide-treated cells.

Taken together, the results discussed above raise two further questions: (*a*) What is the mechanism of this negative control? The labile repressor(s) could decrease the activity of a trans-acting factor activating transcription of the three genes; alternatively, it could exert its effect by binding to a common DNA sequence in the vicinity of the three genes. In this context, it is interesting to note that many macrophage products that are part of the inflammatory response, including TNF-α and IL-1, share a conserved sequence (UUAUUUAU) in the 3′ untranslated region of their mRNAs (21). This sequence is also present in the 3′ untranslated region of the u-PA gene (position 2,239) (5), and in that of the c-*fos* gene (21). Transcription of these four genes is enhanced by cycloheximide; thus, the conserved sequence could be part of the repressor binding site. (*b*) Is the action of IFN-γ exerted through inactivation of labile repressors? The observation that IFN-γ decreases transcription of the c-*fos* gene, which is increased by cycloheximide, argues against this possibility. In any event, the effects of IFN-γ and protein synthesis inhibition show that complex regulatory networks modulate the transcription of genes essential to macrophage activity.

Summary

Exposure of mouse resident and thioglycollate-elicited peritoneal macrophages to IFN-γ leads to a marked increase in the TNF-α (tumor necrosis factor/cachectin), IL-1 and u-PA (urokinase-type plasminogen activator) mRNA levels. Nuclear run-on experiments show that IFN-γ acts by enhancing the transcription of these three genes. Transcription of these three genes is also rapidly and transiently induced by cycloheximide, an inhibitor of protein synthesis, indicating that they are under the control of short-lived repressors.

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