Posttranscriptional Regulation of Interferon mRNA Levels in Peritoneal Macrophages

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Low levels of beta interferon (IFN) mRNA are transcribed in freshly explanted murine peritoneal macrophages. Nuclear runoff transcription assays show that this “constitutive” IFN-β mRNA transcription does not increase in macrophages treated either with lipopolysaccharide or with IFN-γ, which markedly increase this mRNA levels and greatly increase IFN secretion. Therefore, these agents promote accumulation of IFN-β mRNA by posttranscriptional mechanisms. The IFN-α2 gene is also constitutively transcribed by macrophages, but the corresponding mRNA does not accumulate in lipopolysaccharide-treated cells.

Unstimulated peritoneal macrophages explanted from normal mice express small amounts of interferon (IFN) in amounts that maintain these cells in an antiviral state (2, 3, 12, 16). We have recently reported that low levels of IFN-β mRNA can be detected in these macrophages (7). Bacterial lipopolysaccharide (LPS) and IFN-γ increase the level of mRNA in macrophages and induce secretion of IFN-γ in the culture medium (7). There is presently no satisfactory explanation for the “constitutive” IFN-β production by peritoneal macrophages, but it has been suggested that LPS may be involved in stimulating IFN synthesis in vivo (8, 19). Therefore, it is relevant to establish whether LPS and IFN-γ increase the rate of transcription of IFN-β mRNA. We have measured transcription of IFN genes by nuclear runoff assays and found that unstimulated peritoneal macrophages constitutively transcribe IFN-α and IFN-β mRNA; this rate of transcription is not increased by in vitro treatment with LPS or IFN-γ. These agents presumably act at a posttranscriptional level by specifically stabilizing IFN-β mRNA, since IFN-α mRNA does not appear to accumulate in macrophages.

For each experiment, peritoneal macrophages were obtained from 30 C3H/HeN mice (5 to 8 weeks old; Charles River, Milan, Italy) by washing the peritoneal cavity as described previously (7). The macrophages were incubated for 1 h at 37°C in plastic dishes, and nonadherent cells were removed with three washes. Subsequent treatments with different agents are described in the figure legends. Nuclear runoff transcription assays were carried out by the method of Friedman et al. (6), except that nuclei were prepared by the method of Greenberg and Ziff (11). Briefly, 2 × 107 macrophages were washed with ice-cold phosphate-buffered saline, collected by centrifugation, and lysed in 1 ml of 10 mM NaNCl-3 mM MgCl2-0.5% Nonidet P-40-10 mM Tris buffer, pH 7.4. After 5 min of incubation on ice, the nuclei were pelleted, washed, and resuspended in 0.2 ml of transcription buffer containing the components described previously (11) and 0.1 mCi of [α-32P]UTP. After 30 min of incubation at 26°C, the RNA was isolated (4) and hybridized for 4 days at 37°C to linearized plasmid DNA (3 μg per slot) bound to nitrocellulose. The filters were extensively washed, dried, and then autoradiographed at −80°C. The IFN-β cDNA was a 648 bp BamHI-PstI fragment (14); the IFN-α2 cDNA was prepared as described by Shaw et al. (18); the murine IFN-γ cDNA was obtained from the genomic clone of Gray et al. (10); the glyceraldheyde 3-phosphate dehydrogenase (GAPDH) cDNA was prepared from the clone of Dani et al. (5); the v-fos cDNA was obtained by digestion with PstI of a plasmid obtained from the American Type Culture Collection (no. 41040). The cDNAs were inserted into pGEM-1 plasmids (Promega Biotec). The IFN-α2 cDNA was linearized with EcoRI and transcribed with SP6 RNA polymerase to prepare a riboprobe for hybridization assays carried out as described previously (9). At the end of the incubation, the transcription assay was treated with RNase-free DNase; the riboprobe was phenol extracted and ethanol precipitated before hybridization with 50 μg of total cellular RNA. The samples were then digested for 30 min with RNases A and T1, as described before (9). The RNA was phenol extracted and run on 5% polyacrylamide gels with Tris-borate buffer (9).

We showed previously that LPS and IFN-γ increase the level of IFN-β mRNA in peritoneal macrophages freshly explanted from C3H/HeN mice (7). To establish whether this increase was due to enhanced transcription of IFN-β mRNA, we measured its transcription rate by nuclear runoff assays. Nuclei were isolated from peritoneal macrophages either untreated or treated in vitro with LPS from Escherichia coli (serotype O26:B6; Sigma) and recombinant murine IFN-γ (kindly provided by G. Adolf, Boehringer, Vienna, Austria). Untreated peritoneal macrophages constitutively transcribed IFN-β mRNA (Fig. 1A). Treatment of these cells with LPS (40 μg/ml) for up to 3 h did not increase this transcription rate (Fig. 1A). In other experiments, the macrophages were treated for 15, 30, and 60 min with LPS, but in each case the rate of transcription of IFN-β mRNA was identical to that of untreated controls (data not shown). The transcription of IFN-β mRNA was not increased in macrophages treated for 1 (Fig. 1B), 2, or 4 h with IFN-γ (data not shown). These treatment times were chosen on the basis of our previous observation that the level of IFN-β mRNA increased markedly in peritoneal macrophages 3 h after addition of either LPS or IFN-γ (7).

To show in a positive control that transcription of the IFN-β gene can be induced in cultured macrophages by
appropriate stimuli, we infected these cells with a typical IFN inducer (17), Newcastle disease virus (NDV). Nuclear runoff assays showed that the rate of transcription of IFN-β mRNA was increased after infection with NDV (Fig. 1C). pGEM-1 plasmid DNA was included in the hybridizations as a negative control (Fig. 1). These results indicate that LPS and IFN-γ increase the accumulation of IFN-β mRNA by posttranscriptional mechanisms, presumably by preventing the rapid degradation of this mRNA that is observed in macrophages (15), and suggest that the IFN-β gene can be regulated in macrophages by different mechanisms at both the transcriptional and posttranscriptional levels, depending on the inducing agent.

Zullo et al. (21) have reported that the transcription of c-fos mRNA is increased in virus-infected cells. As a further control that the transcription of specific genes can be induced in cultured macrophages, we showed by nuclear runoff assays that transcription of the c-fos gene was increased in NDV-infected macrophages (Fig. 1C). The rate of transcription of IFN-α2 and IFN-γ mRNA was also measured in control and LPS-treated macrophages by nuclear runoff assays. IFN-α2 is the major mRNA species of the IFN-α gene family transcribed in NDV-infected bone marrow macrophages (15). This mRNA was constitutively transcribed by peritoneal macrophages, but its transcription rate was not increased by treatment with LPS (Fig. 1A). Transcription of IFN-γ mRNA was negligible in both control and LPS-treated macrophages. The background hybridization observed with the IFN-γ probe was comparable to that observed in the negative control with pGEM-1 cDNA (Fig. 1A). As a positive control, the transcription of mRNA for a glycolytic enzyme, GAPDH, was also measured and found to be unchanged in macrophages treated with LPS or IFN-γ (Fig. 1).

We failed to detect IFN-α2 mRNA by hybridizing the RNA extracted from control and LPS-treated macrophages to a riboprobe complementary to this mRNA (Fig. 2). Therefore, transcription of IFN-α2 mRNA did not result in its accumulation in macrophages. In a positive control, IFN-α2 mRNA was readily detected in NDV-infected macrophages (Fig. 2). This finding may be explained by a marked instability of IFN-α2 mRNA in macrophages. It should be pointed out that the hybridization in solution to the riboprobe was carried out under stringent conditions that make this assay quite specific for IFN-α2 mRNA. In contrast, other transcripts of the IFN-α gene family may hybridize to the cDNA probe used in the nuclear runoff assays. However, it is unlikely that any IFN-α mRNA species accumulates in macrophages treated with LPS since neutralization assays with monoclonal antibodies show that these cells secrete mainly IFN-β (20).

The accumulation of IFN-β mRNA induced by IFN-γ and by LPS in macrophages is blocked by an inhibitor of protein kinase C (PKC), staurosporine (7). This finding suggests that PKC activity is necessary for the accumulation of IFN-β mRNA. However, it is not known whether PKC is involved in stimulating transcription of this mRNA. To investigate the role of PKC, C3H/HeN macrophages were treated for 1 h with 3 nM staurosporine before preparation and labeling of nuclei for runoff transcription assays. This treatment was previously shown to inhibit completely the accumulation of IFN-β mRNA (7). The rate of transcription of IFN-α and IFN-β mRNA in staurosporine-treated macrophages was similar to that of untreated controls (Fig. 3). This finding indicates that staurosporine inhibits the posttranscriptional mechanism that leads to accumulation of IFN-β mRNA.

Constitutive transcription in unstimulated macrophages results in the accumulation of very small amounts of IFN-β

FIG. 1. Runoff transcription assays with nuclei of peritoneal macrophages treated with LPS and IFN-γ or infected with NDV. Nuclei were prepared from macrophages treated for 3 h with LPS (40 μg/ml) (A); treated for 1 h with IFN-γ (50 U/ml) (B); or infected for 4 h with 0.5 hemagglutination units of NDV per cell (C). The nuclear RNA was hybridized to cDNA from different plasmids, as described in the text. pGEM is the pGEM-1 plasmid used as a control for non-specific hybridization. IFN-α is a plasmid containing the cDNA of IFN-α2 mRNA.

FIG. 2. Analysis of IFN-α2 mRNA in macrophages. Cellular RNA was hybridized to a riboprobe complementary to IFN-α2 mRNA and fractionated on a 5% polyacrylamide gel. The treatment of the macrophages is indicated: control, untreated; LPS, treated for 3 h with LPS (40 μg/ml); NDV, infected with NDV for 3 h as described in the legend to Fig. 1. The track labeled IFN-α2 shows the duplex formed by annealing the riboprobe to complementary sense RNA transcribed from the plasmid pGEM-IFN-α2 with T7 RNA polymerase.

FIG. 3. Runoff transcription assays with nuclei of peritoneal macrophages untreated or treated for 1 h with 3 nM staurosporine before stimulation with LPS or IFN-γ, as described in the legend to Fig. 1.
mRNA, suggesting that this template undergoes rapid turn-over. Even in NDV-infected macrophages, Hoss-Homfeld et al. (15) have estimated a half-life of 15 min for IFN-β mRNA, compared with 85 min in NDV-infected L929 cells. We have previously shown that the level of IFN-β mRNA is markedly increased in macrophages but not in L929 cells treated with IFN-γ or LPS (7). The present results show that these agents do not upregulate the transcription of the IFN-β gene. Therefore, the increased accumulation of IFN-β mRNA in IFN-γ or LPS-treated macrophages may be explained by a decreased turnover of this template.

The mechanism by which IFN-β mRNA is degraded (e.g., by the action of a specific nuclease) remains to be clarified. It seems likely that LPS and IFN-γ increase the stability of this mRNA by promoting the modification of preexisting factors, since these agents are active in the presence of the inhibitor of protein synthesis cycloheximide (7). IFN-γ modulates PKC activity in peritoneal macrophages (13), and LPS primes these cells for an enhanced response to PKC activators (1). Moreover, the accumulation of IFN-β mRNA induced by these agents is markedly reduced in the presence of the PKC inhibitor staurosporine (7). This finding suggests that PKC is involved in the stabilization of IFN-β mRNA. In agreement with this hypothesis, activators of PKC, such as phorbol esters and telocidin, promote the accumulation in human fibroblasts of the mRNA for another cytokine, interleukin-1β, by posttranscriptional stabilization of this template (20). A possible explanation for these observations is that PKC regulates a specific nuclease in an unknown way.

The finding that IFN mRNA is degraded much faster in macrophages than in L929 cells (15) suggests that the turnover of IFN mRNA is specifically regulated in different cells. The present results are consistent with a regulatory pathway that results in rapid turnover of IFN-β mRNA in unstimulated macrophages but leads to increased stability of this template in macrophages stimulated by LPS or IFN-γ.

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REFERENCES