We investigated the molecular basis of the synergistic induction by interferon-γ (IFN-γ)/tumor necrosis factor-α (TNF-α) of human interleukin-6 (IL-6) gene in THP-1 monocytic cells, and compared it with the basis of this induction by lipopolysaccharide (LPS). Functional studies with IL-6 promoter demonstrated that three regions are the targets of the IFN-γ and/or TNF-α action, whereas only one of these regions seemed to be implicated in LPS activation. The three regions concerned are: 1) a region between −73 and −36, which is the minimal element inducible by LPS or TNF-α; 2) an element located between −181 and −73, which appeared to regulate the response to IFN-γ and TNF-α negatively; and 3) a distal element upstream of −224, which was inducible by IFN-γ alone. LPS signaling was found to involve NF-κB activation by the p50/p65 heterodimers. Synergistic induction of the IL-6 gene by IFN-γ and TNF-α in monocytic cells, involved cooperation between the IRF-1 and NF-κB p65 homodimers with concomitant removal of the negative effect of the retinoblastoma control element present in the IL-6 promoter. This removal occurred by activation of the constitutive Sp1 factor, whose increased binding activity and phosphorylation were mediated by IFN-γ.

IL-6 is a multifunctional cytokine involved in controlling many cell functions, including antibody synthesis by B cells, T cell cytotoxicity, stem cell differentiation, and induction of acute phase proteins. IL-6 is produced in response to a variety of noxious stimuli, including viral and bacterial infections (1–4). Deficient regulation of the IL-6 gene is involved in the pathogenesis of autoimmune diseases and affects normal and leukemic hematopoiesis (4–9). Recent studies demonstrated the presence of a defect in IL-6 production in Fanconi’s anemia (10, 11), suggesting that this is partly responsible for the altered hematopoiesis in Fanconi’s anemia patients.

The transcriptional regulatory elements present in the 5’-flanking region of the human IL-6 gene have been studied by several laboratories. Various elements responsible for IL-6 gene induction have been identified, including phorbol 12-myristate 13-acetate, cAMP, NF-κB, NF-IL-6, and multiple cytokine-responsive elements (3, 4, 12–16). Tumor suppressor gene products p53 and pRb have been reported to suppress IL-6 promoter activity (17).

Different laboratories, including ours, showed that there are cell type-dependent differences in the mechanism and transcription factors involved in IL-6 gene induction. Thus, TNF-α-induced IL-6 in a variety of cell types (18–21), but it failed to do so in monocytes (2, 3, 20). We have shown that IFN-γ is an essential co-signal for TNF-α in the induction of IL-6 in monocytic THP-1 cells (22).

The role of IL-6 in the immune response, acute phase reaction, and hematopoiesis, and the fact that monocytic cells appeared to be one of the major physiological sources of this cytokine, prompted us to analyze the molecular mechanism responsible for its induction in this cell type.

Although the mechanism of transcriptional activation mediated by different inducers in several cell types has been extensively studied, the mechanism responsible for synergistic IL-6 gene induction by IFN-γ and TNF-α in monocytic cells has still not been identified.

We postulated that both IFN-γ and TNF-α activate transcription factors, which should act simultaneously to induce IL-6 gene expression. This hypothesis was supported by our previous finding that sequential stimulation with IFN-γ, followed by TNF-α stimulation, did not lead to IL-6 mRNA induction (22). This implied that IFN-γ and TNF-α induced or activated different transiently expressed components, which must act together in cooperation to trigger IL-6 gene expression.

It has been suggested that the induction of NF-κB binding activity by TNF-α contributes to the activation of the IL-6 promoter in some cell lines, including monocytic cells (3, 18–20). The NF-κB/Rel family of transcription factors consists of at least five proteins, including p65 (Rel A), p50, c-Rel, and p52, which are related to each other through an N-terminal stretch of 300 amino acids called the Rel homology domain. DNA binding occurs through dimerization of the family members, resulting in numerous homo- and heterodimeric combinations of NF-κB/Rel proteins. The C-terminal region of p65, c-Rel, and Rel-B harbor a transcriptional activation domain, and the in vivo transcriptional activity is attributed to the p65-, c-Rel-, c-Rel,
and Rel-B-containing dimers (23).

The elements involved in the IFN-γ-mediated induction of IL-6 expression are not known. The binding of IFN-γ to its specific receptors rapidly activates a latent cytoplasmic transcription factor, ISGF3 (interferon-stimulated gene factor 3). ISGF3 is transiently activated and has been shown to stimulate ISRE-dependent transcription (24–27). Other regulatory factors, including interferon-regulatory factor 1 (IRF-1) and IRF-2, have also been shown to be involved in the regulation of the IFN system. IRF-1 and IRF-2 bind to similar cis elements within type I IFN and IFN-inducible genes. IRF-1 functions as a transcriptional activator, while IRF-2 represses IRF-1 function (28–33). Both factors appear also to bind specifically to ISRE sequences (34, 35).

We performed a functional analysis of the 5'-flanking region of IL-6 gene using transient transfection of CAT reporter gene linked to IL-6 promoter, in order to clarify the molecular mechanism involved in the synergistic induction by IFN-γ and TNF-α of the IL-6 gene in human monocytes. The monocytic THP-1 cell model appears to be particularly suitable for molecular dissection of IFN-γ/TNF-α-mediated synergistic induction of IL-6 gene expression, because in these cells, the IL-6 gene is not constitutively expressed (22).

Analysis of IL-6 promoter constructs, electrophoresis mobility shift assay (EMSA), and immunoprecipitation analysis have shown that the synergistic induction of IL-6 gene by IFN-γ and TNF-α in human monocytic cells involved cooperation between IRF-1 and NFκB binding elements, as well as the removal of the negative effect of the retinoblastoma control element (RCE) present in the IL-6 promoter (IL-6-RCE). This IL-6-RCE contains, among other components, a core 5′-CCGCC-3′ sequence homologous with the consensus binding motif, which is the target of the Sp1 factor. Sp1 is a constitutively expressed transcription factor present in a wide range of cell types and binds a GC-rich consensus sequence present in many cellular and viral promoters. Sp1 contains three zinc fingers that mediate DNA binding and four domains that mediate transcriptional activation (36). Upon binding to the DNA containing the GC box in the cell nucleus, Sp1 becomes phosphorylated on multiple sites by double-stranded DNA-dependent kinase (37). It has been shown that specific interaction between DNA-binding domains of the p65 subunit-NFκB and Sp1 bond the DNA modulates transcription of human immunodeficiency virus type 1 in response to cellular activation (38).

Our results show that the contribution of IFN-γ to the triggering of IL-6 gene expression in human monocytes involves a change in the amount and phosphorylated state of Sp1, together with the induction and activation of IRF-1. These factors cooperate synergistically with homodimer p65-NFκB, which is activated by TNF-α.

Role of IRF-1, NFκB, and Sp1 in Human IL-6 Gene Regulation

IL-6-CAT Construct and the 5′-Deletion Mutants—A 1.2-kilobase pair BamHI-XhoI fragment which contains the IL-6-5′-flanking region of promoter (18) was deleted by using the Exo-mung deletion kit (Stratagene), inserted into a pCAT™-Basic plasmid (Promega) at the SalI blunt end and HindIII sites, resulting in the following plasmids: del(-181) (-181 to +14), del(-108) (-108 to +14), del(-73) (-73 to +14), del(-67) (-67 to +14), del(-60) (-60 to +14), del(-54) (-54 to +14), del(-36) (-36 to +14), del(-22) (-22 to +14) and del(-1) (-1 to +14). A del(-36) to -14 plasmid was generated by deleting the BamHI-NdeI fragment (-1160 to -224). All resultant plasmids were verified by sequencing.

Transient Transfections—2 × 10⁵ THP-1 cells (logarithmic growth phase, washed three times with PBS) suspended in 200 μl of DMEM (Dulbecco’s minimal essential medium, Life Technologies, Inc.) were mixed with 5 μg of each of the above constructions, 4 μg of pCATβ-galactosidase control plasmid (Promega) and 5 μg of p53 promoter plasmids and 5 μg of pSVβ-galactosidase control plasmid as an internal reference (Promega) (purified by two cycles of CsCl gradient) and were electroporated at 220 V, 960 microfarads, for 42 ms using a Gene Pulser wired to an electroporation chamber (Bio-Rad). Cells were maintained at room temperature for 10 min before dilution in 10 ml of prewarmed RPMI 1640 supplemented with 10% FCS. Cells were stimulated 1 h later with IFN-γ (400 units/ml) and/or TNF-α (400 units/ml), or with LPS (1 μg/ml) in fresh RPMI 1640/10% FCS. After stimulation of 36 h, cells were analyzed for CAT activity, essentially as described by Gorman et al. (39). Each CAT reaction was performed with 20 μg protein extract and 2.3 nmol of 32P-labeled chloramphenicol (54 mCi/mmol) for 2 h at 37 °C. β-Galactosidase enzyme was measured in the same cell extracts as described previously (40).

Nuclear Extracts and EMSA—To prepare nuclear extracts, 4 × 10⁶ cells were washed twice in chilled PBS and then resuspended in 500 μl of lysis buffer containing 10 mHepes (pH 7.9), 50 mM NaCl, 1 mM EDTA, 5 mM MgCl₂, 10 mM sodium orthovanadate, 10 mM sodium molybdate, 0.2 mM phenylmethylsulfonil fluoride, 10 μM protease inhibitors (pepsatin, leupeptin, aproatin), and 0.05% Nonidet P-40. After swelling for 20 min on ice, gel was added (final concentration 5%, v/v) and nuclei were pelleted by centrifugation at 600 rpm for 10 min, at 2 °C. After rapid washing with the same buffer (containing 140 mM NaCl instead of 50 mM), the nuclei were gently resuspended in 100 μl of storage buffer containing 10 mHepes pH 7.9, 400 mM NaCl, 0.1% IGEPAL CA-630, 5 mM glycerol, 5% glycerol, 2 mM dithiothreitol, 5 mM phenylmethylsulfonil fluoride, 10 mM sodium vanadate, 10 mM sodium molybdate, and 10 μM protease inhibitors. After 60 min on ice with gentle (and occasional) mixing, particulate matter was eliminated by centrifugation at 100,000 × g for 10 min at 4 °C. Protein content in the supernatant was determined using Bradford’s method (81).

For EMSA, nuclear protein (10 μg) was incubated with radiolabeled probe (20,000 cpm) in buffer 10A (10 μg/mL NaCl, 10 mM Hepes (pH 7.9), 5 mM Tris-HCl (pH 7.9), 1 mM dithiothreitol, 15 mM EDTA, 10% glycerol, 500 μg/mL BSA-FV, and 800 μg/mL denatured salmon sperm DNA (in a final volume of 12.5 μl) (without EDTA in the case of Sp1 oligonucleotide probe). After a 30-min incubation on ice, the nucleoprotein complexes were resolved by a nondenaturing electrophoresis in a 5% polyacrylamide gel for 2 h at 20 mA in 1 × TGE buffer (50 mM Tris-HCl (pH 8.8), 180 mM glycine, and 2.5 mM EDTA). In refraction condition. The gel was dried and exposed overnight to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA). For competition experiments, a 100-fold molar excess of the unlabeled oligonucleotides was added 15 min before incubation of nuclear extracts with the end-labeled oligonucleotides, while antisera were mixed directly with nuclear extracts and binding buffer (without salmon sperm DNA) 1 h before adding salmon sperm DNA and radiolabeled probe.

Immunoprecipitation and Immunoblot Analysis—Nuclear protein samples (150 μg) were preclared with rabbit IgG nonimmune antisera and protein A-Sepharose (PharMaco) 2 h at 4 °C. After centrifugation (10,000 × g) the supernatants were incubated with antiserum for 4 h at 4 °C (1500× dilution) overnight at 4 °C, and then 8 μl of protein G-Sepharose (PharMaco) was added and gently rocked for 4 h at 4 °C. Protein A- and protein G-Sepharose were swollen in 50 mM Tris-HCl (pH 8.8), 500 mM KCl, 1% Triton X-100, 0.5 mM phenylmethylsulfonil fluoride, 10 μM protease inhibitors, 10 mM sodium molybdate, 10 mM sodium orthovanadate. The immunoprecipitates were successively washed three times with 100 mM Tris-HCl (pH 8.8), 500 mM NaCl, 1% Triton X-100, Once with 10 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 10% glycerol, and once with 10 mM Tris-HCl (pH 8.8). All washes were performed with buffers containing the anti-proteases and anti-phosphatase mixture. The proteins were eluted in 50 μl of 3% SDS, 30% glycerol, 150 mM KCl, 10 mM Tris-HCl (pH 6.7), 200 mM β-mercaptoethanol (10 min at 95 °C), and specific immunoprotein complexes were separated by SDS-polyacrylamide gel electrophoresis, transferred to a

MATERIALS AND METHODS

Cell Culture—THP-1 cells (strain TB202; American Type Culture Collection, Rockville, MD) were grown (7% CO₂) in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% heat-inactivated FCS shown to be endotoxin-free (<0.1 IU/ml; Myodone, Life Technologies, Inc.).

Reagents—HuHNF-α (specific activity, 2 × 10⁹ units/mg protein) was a gift from Roussel-Uclaf (Romainville, France); HuHNF-α was provided by Shering (Kenilworth, NJ); HuHNF-α (specific activity 6 × 10⁹ units/mg protein) was produced by Genentech and provided by Boehringer-Ingehelm (Dr. G. R. Adolf, Vienna, Austria); LPS (Escherichia coli, serotype O111:B4) was purchased from Sigma. Rabbit affinity-purified polyclonal antibodies against human Rb, Sp1, IRF-1, IRF-2, and NFκB family proteins were from Santa Cruz Biotechnology, Inc. (Tebu, France). Monoclonal anti-phosphoserine was from Sigma. Purified Sp1 protein was from Promega. Enhanced chemiluminescence (ECL) Western blotting kit reagent and Rabbit reticulocyte lysate system were from Amersham (Les Ulis, France).
Role of IRF-1, NFκB, and Sp1 in Human IL-6 Gene Regulation

The results of the deletion analysis described above suggested the presence of at least three regions differentially involved in IL-6 gene regulation by IFN-γ and/or TNF-α, and by LPS. The first region is a distal fragment between −1200 to −1224 that positively regulates the response to IFN-γ alone. Although deletion of this fragment does not eliminate the synergistic IFN-γ + TNF-α response, a slide reduction in the magnitude of the induction of the CAT activity is observed. Computer-based observation showed that this fragment contains an AP-1 site between −283 and −277, and a copy of the IFN enhancer core sequence 5′-AAAGGA-3′ (253–248) (13). The second region is a repressor element, i.e., the sequence between −181 and −73, which would negatively affect the synergistic response to IFN-γ and TNF-α without affecting sensitivity to LPS. This IL-6 DNA sequence contains, from −126 to −101, a direct repeat which is strikingly similar to the c-fos basal transcription element, with 21/26 nucleotides matching the RB-repressible RCE (the RB control element) identified in the c-fos gene (17, 46). In this connection, Santhanan et al. (17) showed in functional assays that the overexpression of wild type RB in Hela cells strongly repressed the activity of IL-6 promoter constructs. In addition to these two elements, a third region located between −73 and −36, probably corresponding to the minimal element necessary for the LPS response, also allowed TNF-α sensitivity. Within this fragment were a putative AP-1 motif (−61 to −55) and the sequence 5′-GGGATTTC-3′ (−72 to −63), which is highly homologous to the immunoglobin κ light-chain enhancer sequence 5′-GGGG-ACCTTCC-3′ (42).

Activation of Nuclear Protein(s) Binding to the IL-6 Promoter DNA Fragments after Stimulation of THP-1 Cells—To determine whether IFN-γ and/or TNF-α induced the binding of factors that specifically recognize DNA sequences in the IL-6 promoter, we prepared double-stranded synthetic oligonucleotides corresponding to IL-6 promoter DNA motif targets of specific transacting factors reported to be involved in the IL-6 gene induction in other cell types (3, 12–16). Nuclear protein extracts, prepared from both untreated cells...
and cells stimulated for 1 h with either IFN-γ and/or TNF-α or with LPS, were submitted to EMSA with several radiolabeled double-stranded synthetic oligonucleotides. This experiment was an initial experiment, which was designed to screen the early induction of specific protein-DNA complexes.

Fig. 2 shows an EMSA using five different oligonucleotides corresponding to (i) NFκB-like and AP-1 binding motifs (A/−273), (ii) IL-6-RCE (RB control element) target motif (B/−126), (iii) the typical cAMP/phorbol ester-responsive motif, and the IL-1/TNF-α responsive elements (C/−173), (iv) the negative regulatory domain-like sequence (and two putative copies of GGAA motifs considered to be responsible for IFN inducibility (D/−207), and (v) the consensus AP-1 motif and a copy of the IFN enhancer core sequence 5′-AAAGGA-3′ (E/−283) (13).

Formation of protein-DNA complexes was apparent with the three regions A, B, and E described above, which were involved in the functional response of the 5′-flanking regions of the IL-6 gene (Fig. 2). Surprisingly, no specific complexes were observed with regions C and D, previously reported to be involved in IL-6 gene regulation by various inducers in other cells systems (3, 4, 12–16).

Induction of NFκB Binding Activity by TNF-α in THP-1 Cells without Concomitant IL-6 Production—We investigated the kinetics of NFκB activation in IFN-γ and/or TNF-α-treated THP-1 cells, and LPS-treated cells. Cells were stimulated by the various inducers for 0.5, 1, or 2 h and nuclear extracts analyzed for binding to the NFκB motif, using the radiolabeled synthetic oligonucleotide A/−73 (Fig. 3). Activated complexes were detectable within 1 h after stimulation with TNF-α alone, as well as with LPS (Fig. 3A). The binding activity shown after combined treatment with IFN-γ and TNF-α was similar to that obtained after treatment with TNF-α alone. The abundance of the complexes induced by TNF-α alone or combined with IFN-γ and by LPS increased throughout 2 h of stimulation without complex mobility change (Fig. 3A). After LPS treatment, the amount of the complexes increased throughout 4 h of stimulation; in contrast, TNF-α either alone or combined with IFN-γ, led to a transient increase for 2 h and dramatically decreased thereafter (data not shown). The specificity of the protein-DNA complexes was confirmed by complete competition of the binding with unlabeled oligonucleotide A/−273 as well as with the immunoglobulin NFκB consensus motif, and the absence of competition with AP-1 or SP1 consensus oligomers (Fig. 3B).

We did not observe any specific binding complexes in untreated cells or in cells stimulated with either IFN-γ or IFN-α.

Polyclonal rabbit antibodies against p50 and p65 subunits of NFκB were used to probe the nuclear IL-6-NFκB binding complexes for the presence of corresponding proteins (Fig. 3B). Addition of antisera against the p65-NFκB subunit supershifted the protein-DNA complexes induced by TNF-α but im-
paired the formation of the complexes induced by LPS. Addition of antisera against the NFκB subunit p50 did not modify the NFκB-induced complexes, but those induced by LPS were partially supershifted. Antibodies against the p52 and c-Rel NFκB subunits did not interact with any of these protein-DNA complexes. Nevertheless, combined treatment with antibodies against p65 + p52 + c-Rel NFκB subunits appeared to result in the disappearance of the minor residual complex left in TNF-α-treated cell extracts (data not shown). Results similar to those shown for the TNF-α-induced complexes were obtained using nuclear extracts of cells stimulated by IFN-γ + TNF-α. These results suggest that p65 is contained in the complexes, whatever the inducers used, whereas p50 only seems to be a constituent of the protein-DNA complexes induced by LPS.

To further investigate the subunit composition of the nuclear IL-6-NFκB binding complexes, nuclear extracts were immunoprecipitated with specific antibodies, and analyzed by Western blot (Fig. 3C). In agreement with the results shown in Fig. 3B, p65 subunit-NFκB was found after stimulation with TNF-α alone, with IFN-γ + TNF-α and LPS, whereas antibody against p50 only revealed a major protein of 50 kDa after LPS stimulation (Fig. 3C).

We previously showed that in THP-1 cells, stimulation by combined treatment with IFN-γ and TNF-α was required for endogenous IL-6 gene expression and protein secretion, and that TNF-α treatment alone was ineffective (22). Here, however, TNF-α was effective in activating nuclear protein binding to the NFκB-like element and in driving CAT expression after transfection with the del(-73) construct containing the NFκB-like sequence. These observations suggested the presence of a repressive element(s) that might negatively affect TNF-α-mediated transcription of the endogenous IL-6 gene.

Involvement of RCE (Retinoblastoma Control Element) in IL-6 Gene Repression in THP-1 Cells: Role of SP1 Protein—It was reported that Rb protein but not its mutants, repress IL-6 promoter transcriptional activity in NIH 3T3 cells (17). This repression was mediated through a cis-acting element, first described in the c-fos promoter (46). The IL-6 promoter contains a G+C-rich sequence between −126 and −104 that includes two CCACC motifs (−123 to −118 and −109 to −103) previously identified as the RB response element (RCE), and one CCGCC motif similar to the Sp1-binding site (−119 to −115) (12, 13).

The results shown in Fig. 1 suggest that a cis-DNA element, which is located between −181 and −73 and contains, among others, the RCE motifs, might be involved in repression of IL-6 gene expression in THP-1 cells. These observations prompted us to investigate the interactions between proteins and the IL-6-RCE sequence in nuclear extracts of THP-1 cells stimulated with various inducers. For this investigation, we used an oligonucleotide spanning both sites (B/−126). EMSA revealed the presence of a major constitutive complex in untreated cells (Fig. 4A, left section). No major modification was observed after this stimulation. Complex formation was specific, since it competed with the non-radioabeled specific oligonucleotide but not with the unrelated oligonucleotide AP-1 (Fig. 4C). Note that protein-DNA interactions appeared to involve the Sp1 elements, since binding displayed complete competition with a
Role of IRF-1, NFκB, and Sp1 in Human IL-6 Gene Regulation

Sp1 consensus motif (Fig. 4B).

Antibodies against purified Sp1 protein partially supershifted the B/−126 complex, whereas antibodies against Rb protein had no effect on complex formation (Fig. 4B). Furthermore, addition of purified Sp1 protein to the nuclear extracts led to a higher site occupancy without changing mobility (Fig. 4B, right section).

Taken together, these results suggest that Sp1 is part of the protein-DNA complex formed with B/−126 oligonucleotide. This oligonucleotide, which encompasses the RCE–IL-6 promoter element, includes the core sequence 5′-CCGCC-3′ (−119 to −115), which covers part of the RCE motif (−123 to −118); this motif appears to be homologous with the 5′-CCGCC-3′ core sequence, reported to be the consensus binding motif involved in the interaction with the Sp1 factor. Furthermore, when we used an Sp1 consensus oligonucleotide as the radiolabeled DNA probe in EMSA experiments, a major constitutive complex was revealed (Fig. 4A, right section) with a mobility similar to that obtained with the B/−126 probe (Fig. 4A, left section). The amount of the protein-Sp1 oligonucleotide complex rose slightly after stimulation by IFN-γ, TNF-α, LPS, or IFN-α, and rose more markedly after combined stimulation with IFN-γ + TNF-α. Complex formation was impaired by competition with the specific unlabeled Sp1 oligonucleotide, and partial competition was observed with the B/−126 oligonucleotide (Fig. 4C). As expected, antibodies against the Sp1 protein led to a supershift of the protein-DNA complex, whereas no effect was observed with pRb antibodies. In the same way, as for the B/−126 probe complex, addition of Sp1 protein greatly increased the amount of protein-DNA complex revealed with the Sp1 oligonucleotide probe.

To demonstrate the presence of Sp1 protein in the B/−126 DNA protein complex, we used shift-Western blot analysis, with either the B/−126 probe or the Sp1 consensus probe. When a complex was formed with the B/−126 probe (Fig. 5, A1), we could not reveal the presence of Rb protein (A2), whereas the antibodies against Sp1 protein recognized a specific protein whose abundance increased slightly after IFN-γ + TNF-α treatment (A3). The antibodies against Rb protein identified a faint lower band, probably corresponding to free Rb protein (Fig. 5, A2).

As expected, in a similar experiment using a radiolabeled Sp1 probe (Fig. 5B), antibodies against Sp1 protein revealed a specific protein contained in the protein-DNA complex (B3); the amount of Sp1 protein correlated with the amount of DNA probe bound to this complex (B1), which increased after IFN-γ + TNF-α treatment (B3). A faint lower band was also seen, probably corresponding to free Sp1 protein. With pRb antibodies, no protein was revealed in the protein-DNA complex, and in the experiment using the B/−126 probe, a lower band was detected, corresponding to free Rb protein (Fig. 5, B2).

Immunoprecipitation of crude nuclear extracts of THP-1 cells stimulated with IFN-γ alone or combined to TNF-α, using antibodies against Sp1 protein, dramatically raised the level of the 95-kDa native Sp1 protein as shown by Western blot analysis (Fig. 4D, upper section). To demonstrate that the effect of IFN-γ or IFN-γ + TNF-α correlated with the activation of Sp1 protein, the same immuno-Western blot was hybridized with a monoclonal anti-phosphoserine antibody. Fig. 4D (lower section) shows an increase in phosphorylated Sp1 protein, previously reported to be the active form of this nuclear factor (36). These results correlate well with the increase in binding activity shown by EMSA performed with the Sp1 probe after THP-1 cell treatment with IFN-γ + TNF-α (Fig. 4A, right section).

Taken together, these results suggest the involvement of the Sp1 protein in the protein-DNA interaction of the RCE present in the IL-6 promoter. Although we cannot exclude the possibility that other factors interact with the RCE–IL-6 promoter, shift-Western blot and antibody-supershift experiments nevertheless suggest that Sp1 is part of the activity that interacts with this region.

Regulation of IRF-1 and IRF-2 in the Synergistic Induction of IL-6 Gene Expression by IFN-γ + TNF-α—Functional analysis (Fig. 1) showed that a distal element between −1200 and −224 regulates positively the response to IFN-γ alone, or combined with TNF-α. EMSA using probe E/−283, and encompassing a copy of the IFN enhancer core sequence, revealed the formation of 2 protein-DNA complexes, one of which was specifically induced by IFN-γ treatment (Fig. 2). We investigated the kinetics of complex activation in THP-1 cells treated with IFN-γ...
Role of IRF-1, NFκB, and Sp1 in Human IL-6 Gene Regulation

**Fig. 4. Specificity of protein binding to RB control elements.** A, EMSA was performed with nuclear extracts of THP-1 cells, either untreated or stimulated for 0.5 h with IFN-γ and/or TNF-α, LPS, or IFN-α, using radiolabeled double-stranded oligonucleotide B/-126 (left section), or radiolabeled consensus Sp1 oligonucleotide (right section). B, nuclear extracts of cells left untreated for 0.5 h (Cont) were submitted to EMSA using the B/-126 radiolabeled probe to reveal specific competition between RCE-binding proteins and either unlabeled double-stranded oligonucleotide B/-126 or Sp1 consensus oligonucleotide. AP-1 unlabeled oligonucleotide was used as unrelated competitor. Nuclear extracts of untreated cells (Cont) or IFN-γ-treated cells were preincubated with antibodies (Ab) against Rb protein (Rb) or against Sp1 protein (Sp1), and radiolabeled probe B/-126 was then added. Recombinant Sp1 protein (1.0 footprinting units) was mixed with nuclear extract of untreated cells (Cont) or cells treated with IFN-γ, and radiolabeled B/-126 probe was then added (right section). C, specificity of the EMSA complexes revealed with radiolabeled probe Sp1. Before the addition of radiolabeled Sp1 oligonucleotide, nuclear extracts of untreated cells or cells treated with IFN-γ, TNF-α, or IFN-γ + TNF-α were mixed either with unlabeled oligonucleotide (-126, Sp1), or with antibodies (Ab) against Sp1 protein or Rb protein; NI corresponded to nonimmune rabbit antiserum. Sp1 protein (1.0 footprinting unit) was added to the same nuclear extracts. D, immunoprecipitation of nuclear extracts of untreated THP-1 cells (Cont) or cells stimulated with IFN-γ alone or IFN-γ + TNF-α, using antibodies against Sp1 protein, followed by Western blot analysis. Upper part shows blot hybridization with Sp1 antibodies. Lower part, the same blot was dehybridized and reprobed with antibodies against phosphoserine protein. Left arrows point to the specific Sp1 protein. Right arrows (mass in kDa) point to the relative mass of protein molecular markers.

**Fig. 5. Sp1 protein is part of IL-6-RCE-protein complex.** EMSA was performed using either the radiolabeled B/-126 oligonucleotide (A) or the radiolabeled Sp1 consensus oligonucleotide (B). Shift-Western blot analysis was then performed as described under “Materials and Methods.” Radiolabeled DNA was visualized on DEAE membrane (A1 and B1) whereas either Rb protein (A2 and B2) or Sp1 protein (A3 and B3) was revealed on nitrocellulose by Western blot analysis using the respective specific antibodies against Rb protein and Sp1 protein.

and/or TNF-α, LPS, and IFN-α. The same nuclear extracts as those already used for the NFκB experiments were analyzed for binding activity to the IFN-enhancer motif, using probe E/-283 (Fig. 6A). In untreated cells, EMSA revealed the presence of a constitutive DNA-protein complex, C1 whose amount increased in a time-dependent manner after stimulation by IFN-γ and IFN-γ + TNF-α. The amount of this C1 complex was not modified by TNF-α, LPS, or IFN-α. Note that, IFN-γ alone
induced a second protein-DNA complex (C2), which was not observed after TNF-α or LPS stimulation. EMSA carried out with nuclear extracts of cells treated with IFN-α for 2 h revealed a faint C2 complex.

To define the sequence specificity of the C1 and C2 complexes, competition experiments were performed with synthetic oligonucleotides representing several mutations of the −283 to −242 region either in the putative interferon enhancer core sequence 5′-AAAGG-3′ (−283/mt31) or in the AP1 motif (−283/mt15), and also with the ISRE of the 2′5′-oligo(A) synthetase gene promoter, known to be implicated in IFN responses (43), and with the C3 oligonucleotide defined by its IRF-1-binding activity (44).

Oligonucleotide E/−283 and the mutant −283/mt15 competed for the formation of both complexes (Fig. 6B, mt 15), whereas the mutant −283/mt31 lost this capacity (Fig. 6B, mt 31), indicating that the IFN enhancer core sequence 5′-AAAGG-3′ was involved in the DNA-protein interaction. Complete competition for the C1 and C2 complexes was observed with the ISRE and C3 oligonucleotides. A trimmer AP1 motif was not able to compete for either complex, in agreement with the behavior of mutant −283/mt15. No competition was observed neither with the Sp1 nor with B/−126 oligonucleotides (Fig. 6B).

To investigate the possible relationship between the C1 and C2 complexes and ISGF3 (25−27, 47), nuclear extracts of THP-1 cells treated for 2 h with IFN-α were tested for their binding activity, using the E/−283, ISRE, or C3 oligonucleotides as DNA radiolabeled probes (Fig. 7). The pattern of the protein-DNA complexes was very similar with the E/−283 and C3 oligonucleotide probes, suggesting that they bind the IRF-1-related factor. For ISRE-DNA-protein binding, only IFN-α treatment resulted in a double upper-induced binding activity (Fig. 7); several constitutive complexes showed no quantitative or qualitative changes, whatever the inducers used. These results suggest that C1 and C2 complexes did not involve ISGF3, but rather IFN-1/IRF-2, unlike the IFN-α-induced complexes revealed with the ISRE probe.

As shown in Fig. 6C, the amount of constitutive DNA-binding C1 complex was markedly reduced by the IRF-2 antibodies, while the inducible complex C2 was supershifted by the IRF-1 antibodies. The IRF-1 antibodies also abolished the time-dependent increase in the C1 complex, which remained at its constitutive level. These results demonstrate that IRF-2 is involved in the formation of the constitutive C1 complex, and that IRF-1 is involved both in the formation of the inducible C2 complex and in the increase in the amount of C1 complex.

To establish whether this typical IRF recognition sequence is sufficient for IFN-γ activation and for the synergistic action of IFN-γ with TNF-α, we constructed mutated IL-6 promoter CAT expression plasmids. For this purpose, we linked oligonucleotide E/−283, or oligonucleotide −283/mt31, which carried a mutation in the putative IFN enhancer core sequence AAAGGA (−253 to −248), to del(−224)-CAT and called the resulting constructs del(−224)E and del(−224)Emt31, respectively (Fig. 1A). These constructs were then transfected into THP-1 cells. As shown in Fig. 1B, the relative induction of CAT activity after THP-1 transfection with construct del(−224)E demonstrated that the IFN enhancer core sequence is sufficient to confer responsiveness to IFN-γ, because the mutation of the AAAGGA motif (see del(−224)Emt31) abolished IFN-γ sensitivity; the IFN-γ + TNF-α synergistic induction was preserved, although to a much smaller extent (2-fold induction). These results suggest that in addition to the IFN enhancer core sequence, surrounding sequences might contribute to the synergistic effect of IFN-γ with TNF-α.

Transfection of either of the constructs del(−224)E or del(−224)Emt31 resulted in a similar LPS-induced increase in CAT activity (7- and 6-fold, respectively), showing that the IFN enhancer core sequence did not play an essential role in LPS induction (Fig. 1). However, LPS inducibility required the cooperation of other(s) factor(s), as demonstrated by the progressive reduction of CAT activity after the transfection of IL-6 promoter 5′ end successive deletions.

These results showed that IFN-γ + TNF-α and LPS induced the IL-6 gene by different pathways.
Differential Regulation of IRF-1 and IRF-2 Expression by IFN-γ or LPS—Previous reports have shown that the IRF-1 and IRF-2 factors display antagonist activities, and that the up-regulation of the binding activity of one factor results in the down-regulation of the binding activity of the other (28–32). Our demonstration that the binding activities of IRF-1 and IRF-2 correlated with the synergistic induction of IL-6 promoter by IFN-γ + TNF-α (Fig. 6) prompted us to compare the regulation of IRF-1 and IRF-2 by IFN-γ, TNF-α, or IFN-γ + TNF-α with their regulation by IFN-α.

For this purpose immuno-Western blot analysis of nuclear extracts of untreated THP-1 cells after 2 h of stimulation was performed using antibodies against IRF-1 or IRF-2. A polypeptide of 42 kDa was revealed by the IRF-1 antibodies, but only after stimulation with IFN-γ antibodies against IRF-1 or IRF-2. A polypeptide of 42 kDa was not modified after cell treatment with IFN-α. IRF-2 antibodies detected a constitutive IRF-2 protein whose abundance was not modified after cell treatment with IFN-γ or TNF-α either alone or combined for 2 h. In contrast, treatment with LPS or IFN-α reduced the amount of IRF-2 protein (Fig. 8A, upper section). The IRF-2 antibodies detected a constitutive IRF-2 protein whose abundance was not modified after cell treatment with IFN-γ or TNF-α either alone or combined for 2 h. In contrast, treatment with LPS or IFN-α reduced the amount of IRF-2 protein (Fig. 8A, left section). Since formation of the constitutive C1 complex involved IRF-2 protein binding (see Fig. 6C), we attempted to establish whether a transient modulation of this protein occurred soon after stimulation. As shown in Fig. 8A (left section), treatment with IFN-γ + TNF-α, LPS, or IFN-α reduced the level of IRF-2 protein in nuclear extracts of cells stimulated for 30 min. The effect of IFN-γ + TNF-α was transient, because the reduction in the amount of IRF-2 protein was not seen in nuclear extracts of cells that had been stimulated for 2 h. In contrast, the inhibition by IFN-α or LPS of the IRF-2 protein level lasted throughout the 2 h of stimulation.

Since the amount of IRF-2 protein diminished after cell stimulation by IFN-γ + TNF-α, LPS, or IFN-α, whereas IRF-1 protein was only induced by IFN-γ, we explored this effect to see if it was related to an increase in IRF-1 and/or a decrease in IRF-2 gene expressions. For this purpose, total RNAs were extracted after 0.5, 1, or 2 h from cells stimulated with various inducers and then analyzed by Northern blot. As shown in Fig. 9, IFN-γ already induced IRF-1 mRNA after 30 min, in an amount which increased for up to 2 h of cell stimulation. No synergistic effect was observed after combined treatment with IFN-γ and TNF-α. Stimulation by IFN-α also triggered IRF-1 gene expression, but with different kinetics of accumulation since IRF-1 mRNA was barely detected after 1 h of IFN-α stimulation but its amount increased thereafter. Two IRF-1 mRNAs of 2.1 and 3 kilobases were revealed, with a similar accumulation kinetic. Neither TNF-α nor LPS induced IRF-1 gene expression in THP-1 cells.

In contrast, IRF-2 mRNA was constitutively expressed (Fig. 9). No significant modification of the IRF-2 mRNA level was observed whatever the period of incubation and the inducers used. The reduction in the level of this protein after stimulations with various inducers, without concomitant decrease in
the IRF-2 mRNA level, prompted us to make sure that IRF-2 mRNA was functionally active. Accordingly, total RNAs were extracted from cells left untreated for 2 h or stimulated for 2 h and translated in vitro, using reticulocyte lysate and [35S]methionine. Identical number of counts/min (5 × 10⁶ cpm) were analyzed by immuno-Western blot, using specific IRF-2 antibodies. Similar amounts of IRF-2 protein were detected whatever the inducers used (Fig. 8B).

These results indicate that the differential regulation of IRF-2 protein by IFN-α, LPS, and IFN-α takes place at post-translational level.

DISCUSSION

We investigated the molecular basis for the IFN-γ/TNF-α-mediated synergistic induction of the human IL-6 gene in the THP-1 monocytic cell line by functional analysis of this gene's promoter. The results provided evidence that three regions inside this promoter are the targets of the IFN-γ/TNF-α action: (i) a region between −73 and −36, which constitutes the minimal element inducible by LPS or TNF-α; (ii) an element located between −181 and −73, which appears to regulate negatively the response to IFN-γ and TNF-α; and (iii) a distal element upstream of −224, which is the only one inducible by IFN-γ alone.

Each of the DNA elements corresponding to the three functional regions inducible by IFN-γ and/or TNF-α and by LPS led to the formation of specific DNA-protein complexes.

The IL-6 promoter DNA fragment between −73 and −36 was found to contain an NFκB-like element near a putative AP-1 binding motif. In THP-1 cells, TNF-α or LPS induced binding to this DNA motif, whereas IFN-γ neither induced NFκB binding activity nor amplified the effect of TNF-α when added together with it. The EMSA-DNA competition experiments with consensus immunoglobulin κB oligonucleotide allowed us to conclude that the formation of the complexes induced by TNF-α and LPS is due to NFκB binding factors. EMSA including antibodies and immunoprecipitation with anti-p50 and anti-p65 NFκB subunit antibodies revealed only the nuclear accumulation of p50 and p65 after stimulation by LPS. The predominance of the p65 subunit was shown to be the major factor induced by TNF-α or IFN-γ + TNF-α. Various dimer combinations of induced factors have been reported to be stimulus-dependent and to display distinct DNA binding specificities by activating distinct sets of target genes (23). Thus, homodimer p65 was previously shown to be selectively activated in other systems by TNF-α (48, 49).

In THP-1 cells, the induction of NFκB binding activity by TNF-α did not correlate with concomitant IL-6 production (22), but required the addition of IFN-γ, unlike activation by LPS. This indicated that the mechanism involved in the induction of endogenous IL-6 gene in THP-1 cells is inducer-specific.

Although NFκB binding factors are certainly involved in IL-6 gene induction, the functional analysis of IL-6-CAT construct deletions reported here shows that significantly greater stimulation of the CAT reporter gene is obtained for constructs that contain the 5′ distal sequence of IL-6 promoter (pR-1200). Successive deletions of the 5′ end of the IL-6 promoter fragment (−1200 to −108) resulted in the gradual reduction of synergistic CAT activity by IFN-γ + TNF-α.

EMSA using the oligonucleotide E−/−283, which contains a copy of IFN-enhancer-core sequence, showed that IFN-γ, but not TNF-α or LPS, induced specific binding activity. Similar results were obtained with C3 oligonucleotide, suggesting that an IRF-1-related factor was a component of the C1- and C2-specific protein-DNA complexes. EMSA experiments using antibodies against IRF-1 or IRF-2 allowed us to conclude that the formation of the constitutive C1 complex involved IRF-2 binding and that IRF-1 was involved, both in the binding activity of the inducible C2 complex and in the increased abundance of the C1 complex due to stimulation by IFN-γ.

In agreement with these findings, transfection of construct del(−224)/Emt31 into THP-1 cells confirmed that the IFN-enhancer-core sequence AAAGG, which is located between −23 and −248 (13), is sufficient to induce the response to IFN-γ alone.

The IRF-1 binding site identified in the IL-6 promoter was shown to be the central motif of the IFN-stimulated response element (ISRE) found in the promoter of IFN-α-stimulated genes such as the 2′,5′-oligo(A)-synthetase gene (43). In EMSA using the E−/−283 probe, the IRF-related complexes C1 and C2 were impaired by the ISRE oligonucleotide. However, after IFN-α treatment, no C1 or C2 binding activity was observed. When EMSA was performed with the ISRE oligonucleotide for purposes of comparison, it only revealed ISGF3-related complexes after IFN-α treatment, as expected (24–27).

Induction of IRF-1 protein-DNA binding by IFN-γ correlated with the induction of IRF-1 mRNA and with the concomitant IRF-1 protein synthesis. On the other hand, IRF-2 mRNA constitutive expression was not modified whatever the inducers, whereas the level of constitutive IRF-2 protein decreased transiently after IFN-γ + TNF-α treatment. LPS or IFN-α treatment led to the disappearance of the constitutive IRF-2 protein.

The different effects exerted on IRF-2 protein down-regulation by IFN-γ + TNF-α on the one hand and by LPS on the other might partly account for the difference between the magnitude of IL-6 induction by each of these inducers in monocyctic THP-1 cells.

LPS was recently shown to induce or activate proteins that recognize the 3′-end instability sequence of an mRNA that prevents the latter's translation (50–52). Such a mechanism might account for the down-regulation of IRF-2 protein in vivo, since here we were unable to show by Northern blot analysis that this inducer modulates either the expression of mRNA IRF-2 or its translation in vitro. Alternatively, the constitutive IRF-2 protein might be rapidly degraded after its activation by IFN-γ or LPS, as shown in other systems (53), thus allowing the binding of another activator which in the case of IFN-γ would be IRF-1.

As LPS did not, under our experimental conditions, induce IRF-1, we might be justified in assuming that one or several other factors activated by LPS could act as a transcriptional factor, as suggested by our functional CAT analysis (Fig. 1B).

Our results suggest that IFR-1 may be a critical downstream signaling factor involved in IFN-γ signal transduction in monocytes/macrophages, particularly for genes whose maximal expression is triggered by combined treatment with IFN-γ + TNF-α (22, 54–56).

The fact that TNF-α induced NFκB binding activity, which correlated with the inducibility of the del(−73)-CAT construct but failed to induce reporter constructs containing additional upstream sequences, suggests that a silencer of NFκB activity must be present in the IL-6 promoter. Functional analysis of deletion constructs indicated that this negative element might be located between −181 and −73. This region contains, among others, a retinoic acid core element known to be involved in pRb-mediated repression of the c-fos promoter (46). The presence, as reported by Santhanam et al., of a region analogous to the RCE in the IL-6 promoter between positions −126/−101, exhibiting similar patterns of c-fos and IL-6 promoters regulation, suggests that RCE may be involved in IL-6 gene repression in monocytic cells. Evidence that RCE has a repressor role was obtained, in NIH 3T3 cells, by deletion experiments and cotransfections of IL-6 promoter with pRB express-
It is tempting to suggest that alteration(s) in the interaction between the putative suppressor factor(s) and RCE allowed us to obtain a synergistic effect of IFN-γ with TNF-α in THP-1 cells. Synergistic CAT induction was observed for del(−181) but not for del(−108). The del(−181)-CAT construct contains the intact IL-6-RCE motif, whereas a truncated RCE motif is present in the del(−108)-CAT construct.

EMSA experiments performed with oligonucleotide B/−126, which contains the IL-6-RCE motif, revealed one major constitutive RCE-protein complex in nuclear extracts of THP-1 cells. This complex was completely inhibited by consensus Sp1 oligonucleotide and was supershifted by Sp1 antibodies. In addition, recombinant Sp1 protein increased this site occupancy. Furthermore, we showed by shift-Western blot analysis that Sp1 protein is part of the IL-6-RCE-protein complex, whereas Rb protein, undetectable in this complex, was not.

Our observations are in agreement with the hypothesis proposed by Udvadia et al. that Rb regulates transcription partly by virtue of its ability to interact functionally with RB control proteins, including Sp1 (45, 57–60).

EMSA performed with the Sp1 consensus probe showed that stimulation of THP-1 cells by IFN-γ + TNF-α enhanced the amount of the protein-DNA complex. This result correlates with a marked increase by IFN-γ in the amount of serine-phosphorylated Sp1 protein.

It has been shown that Sp1 is selectively phosphorylated upon binding to its cognate recognition elements on DNA, suggesting that phosphorylation represents an early event in the processes leading to transcriptional activation by Sp1. Phosphorylation of Sp1 is catalyzed by a double-stranded DNA-dependent kinase and requires binding to DNA containing the GC box (34, 61).

It is tempting to postulate that the effect of IFN-γ was partly mediated by increased binding of Sp1 to its target sequence in the IL-6 promoter, with concomitant Sp1 factor activation by specific phosphorylation. This alteration might impair the negative effect mediated by the IL-6-RCE.

On the other hand, NFκB has been shown to synergize with a number of transcription factors, including Sp1 (62, 63). It has been reported that Sp1 specifically interacts with the N-terminal region of Rel A (p65); similarly, Rel A (p65) bound directly to the zinc finger region of Sp1 factor. This interaction is specific, because Rel A did not associate with several other transcription factors known to be zinc finger proteins (36–38).

Since the p65 homodimer induced by TNF-α in THP-1 cells is not, by itself, sufficient to induce IL-6 gene expression, we can postulate that functional interaction between p65 NFκB and the activated-Sp1 bound to the adjacent target Sp1 site contributed to the induction of the IL-6 gene.

Our observations allowed us to conclude that IL-6 gene expression in THP-1 cells by IFN-γ + TNF-α, or by LPS treatment is mediated through different signaling pathways.

LPS signaling was shown to involve interaction with CD14 (64, 65), which triggered the mitogen-activated protein kinase Ras-Raf1-dependent cascade, leading to NFκB phosphorylation and subsequent translocation allowing accumulation of the p50/p65 NFκB heterodimer in the nucleus (23, 66–68). Since this p50/p65 complex was also identified in our cell system, a similar pathway might be responsible for the IL-6 gene expression induced by LPS in THP-1 cells.

In contrast, the synergistic effect of IFN-γ + TNF-α on IL-6 gene induction in THP-1 cells might involve three simultaneous processes. The first process is the activation of NFκB by TNF-α by a different pathway from that of activation by LPS, leading to the activation of the p65 homodimer. This possibility is supported by a recent report showing that, in murine macrophages, TNF-α activates the mitogen-activated protein kinase cascade in a mitogen-activated protein kinase kinase kinase-dependent but c-Raf-1-independent fashion (69). The second process is the induction of IFN-1 by IFN-γ through a signaling pathway involving the activation of Jak1/Jak2 and Stat1 (70–75). The third process is a concomitant change by IFN-γ, in the state of phosphorylation and abundance of the constitutive Sp1 nuclear factor interacting with IL-6-RCE. The phoshospecific kinase implicated in the signaling pathway leading to phosphorylation of Sp1, presumably activated by IFN-γ, remains to be investigated. Transcriptional induction of the IL-6 gene might result from a coordinated effect exerted by factor Sp1 together with IFN-1 and p65 homodimer-NFκB.

The fact that regulation by IFN-γ of the IL-6 gene in human monocytes involved IFN-1 may be more generally related to the tumor suppressor/differentiation properties of IFN-γ (32, 76). Deletion of a chromosomal segment that contains the IFN-1 gene, mapped in chromosome 5q31.1, is very often observed in human leukemia (77). It is therefore possible that an alteration in the balance of IFN-1/IFN-2 might impair optimal physiological induction of IL-6 by IFN-γ/TNF-α in the monocytic cell compartment, leading to abnormal cell maturation and proliferation in certain hematological disorders and to neoplasia.

Alterations in IL-6 production such as overexpression in multiple myeloma and other type of cancers (2–9, 78, 79) or inhibition in Fanconi’s anemia (10, 80) may be suspected to play a role in pathological cell growth and to affect hematopoietic cell differentiation. In this regard, the fact that the triggering of IL-6 gene expression by IFN-γ in monocytic cells required the induction of IFN-1 and involved phosphorylated Sp1 protein may be of physiological relevance, and one of the important homeostatic properties of IFN-γ within the cytokine network.

Acknowledgments—We thank Dr. T. Taniguchi (Osaka, Japan) for the generous gift of IFN-1 and IFN-2 cDNAs, Dr. G. R. Adolf (Boehringer-Ingelheim, Vienna, Austria) for the gift of human TNF-α, and Dr. Lando (Roussel-Uclaf, Romainville, France) for the gift of human IFN-γ. We are grateful to A. Bird for expert secretarial assistance and to C. Sylvestri for valuable technical assistance for plasmid-DNA purifications and cell cultures.

REFERENCES

1. Sehlal, P. B., May, I., and Vilecok, J. (1987) Science 235, 73–74.
2. Revel, M. (1989) Experientia (Basel) 45, 549–557.
3. Sehgal, P. B. (1990) Mol. Biol. Med. 7, 117–130.
4. Hirano, T. (1992) Cell. Immunol. 153, 138–180.
5. Suzuki, T., Moro, T., Tohda, S., Nagata, K., Yamashita, Y., Imai, Y., Aoki, N., Hirashima, K., and Nara, N. (1990) Jpn. Cancer Res. 81, 979–986.
6. Emile, D., Coumarbas, J. R., Raphael, M., Devenger, D., Deleuze, H. J., Gisselbrecht, C., Michiels, F. J., Vandenenne, J., Taga, T., Kishimoto, T., Creveno, M. C., and Galainan, P. (1992) Blood 80, 498–504.
7. Porgador, A., Tsehoval, E., Katz, A., Vadai, E., Revel, M., Feldman, M., and Eisenbach, L. (1992) Cancer Res. 52, 3679–3686.
8. Kloss, S., Sugiyama, H., Ogawa, H., Yanagami, T., Azuma, T., Oka, Y., Miwa, H., Kita, K., Hiraoka, A., Masakado, T., Asa, K., Kyo, T., Dohy, H., Hara, J., Kanaumara, A., and Kishimoto, T. (1994) Blood 84, 2672–2680.
9. Klein, B., Zhang, X. G., Lu, Z. Y., and Bataille, R. (1995) Blood 85, 863–872.
10. Rosselli, F., Sancalou, J., Witzterbin, J., and Moustacchi, E. (1992) Hum. Genet. 89, 42–48.
11. Dendorfer, U., Oettgen, P., and Libermann, T. A. (1994) Mol. Cell. Biol. 14, 4443–4454.
12. Dendorfer, U., Oettgen, P., and Libermann, T. A. (1994) Mol. Cell. Biol. 14, 4443–4454.
13. Kiehntopf, M., Herman, F., and Brach, M. A. (1995) Exp. Med. 181, 793–798.
14. Santhanam, U., Ray, A., and Sehgal, P. B. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7605–7609.
15. Yasukawa, K., Hiraoka, T., Watanabe, Y., Murataki, K., Matsuda, T., Nakai, S., and Kishimoto, T. (1987) EMBO J. 6, 2939–2945.
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*J. Biol. Chem.* 1995, 270:27920-27931.
doi: 10.1074/jbc.270.46.27920

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