

Interleukin-10 Induces Inhibitory C/EBP β through STAT-3 and Represses HIV-1 Transcription in Macrophages

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Pulmonary tuberculosis (TB) has been characterized by inflammation with increased pro- or anti-inflammatory cytokines produced by macrophages. We have reported that IFN produces inhibitory C/EBP β and represses transcription of the HIV-1 LTR in macrophages. STAT-1 and type I IFN receptor knockout mice have macrophages that are defective in IFN signaling, yet LPS stimulation induces inhibitory C/EBP β , demonstrating that other cytokines can induce this repressor. LPS or *Mycobacterium tuberculosis*-derived lipoarabinomannan induce the anti-inflammatory cytokine interleukin (IL)-10, which represses the HIV-1 LTR in differentiated THP-1 macrophages by inducing inhibitory C/EBP β . In contrast, in undifferentiated THP-1 monocytes, IL-10 did not inhibit HIV-1 replication or induce C/EBP β . IL-10 signal transduction uses STAT-3, and macrophages from STAT-3^{-/-} mice fail to produce inhibitory C/EBP β after LPS or IL-10 stimulation. Transfection of STAT-3 into THP-1 cells enhances C/EBP β promoter activity. THP-1 differentiation also increases STAT-3 protein, but not STAT-3 gene transcription, and induces a translational regulator, CUG-binding protein, that was essential for production of C/EBP β . Differentiation induced post-transcriptional regulation is required to produce inhibitory C/EBP β in response to IL-10. Only macrophages are able to repress HIV-1 LTR promoter activity and inhibit viral replication in response to IL-10 or type I IFN.

Keywords: infection; innate immunity; repression; transcription factors

The epidemic of tuberculosis (TB) in patients with AIDS is a major health crisis both in developing and industrialized countries (1, 2). Alveolar macrophages (AM) are the major source of HIV-1 replication during TB in co-infected patients (3, 4). The increase in viral replication and mutation induced by opportunistic infection likely causes acceleration of AIDS (5). The mechanisms regulating HIV-1 replication in AM are therefore central to the understanding of AIDS pathophysiology. This is especially true since AM are long-lived cells that may serve as a reservoir for HIV-1 during suppression of viral replication during highly active antiretroviral therapy (6).

Regulation of the HIV-1 LTR is an important step in the control of viral replication in infected cells. The HIV-1 LTR has three C/EBP sites in its negative regulatory element (7). These

three sites are essential for control of viral replication in macrophages, but not lymphocytes (8). The C/EBP sites bind a number of transcription factors that regulate proinflammatory cytokine expression (9, 10) and viral replication in monocytes/macrophages (11, 12).

C/EBP β is the predominant C/EBP transcription factor family member expressed in unstimulated AM (13). The C/EBP β gene is intronless, but can produce both stimulatory and inhibitory isoforms depending on which translation start site is selected by the ribosome (14). In humans, there is a 37-kD isoform and an inhibitory 16-kD isoform produced from an internal AUG start codon (15). The 16-kD C/EBP β is a dominant-negative transcription factor repressing promoters that contain C/EBP sites if expressed at 20% of the level of the stimulatory isoform (14). Translation of the inhibitory C/EBP β isoform is induced by the RNA-binding protein, referred to as CUG (16). The inhibitory C/EBP β likely recruits co-repressors to the promoter inhibiting transcription (17). Expression of a dominant-negative 16-kD C/EBP β isoform strongly represses the HIV-1 LTR (18) and the TNF- α promoters in monocytes and macrophages (9). Resting AM strongly express inhibitory C/EBP β , which maintains the baseline quiescent state of the lung (13). Production of the inhibitory C/EBP β is an IFN effect in macrophages but not monocytes (19). Differentiation of monocytes to macrophages leads to IFN responsiveness through post-transcriptional induction of interferon response factor (IRF)-9. Targeted deletion of the Type I IFN receptor or STAT-1 can disrupt IFN signaling, with loss of IFN effects and dissemination of intracellular infectious agents (20, 21).

Interleukin (IL)-10 is induced during TB and appears to be associated with anergy and failure to produce a TH-1 response (22, 23). The IL-10 and TGF- β interact with one another to suppress the immune response (24, 25). IL-10 polymorphisms are associated with alteration of active disease in humans, supporting a clinically relevant role of this cytokine in TB (26). Lymphocytes from anergic patients can suppress HIV-1 replication *in vitro* (27). *In vitro* models, however, suggest that IL-10 can contribute to control of mycobacterial growth in dendritic cells (28). The anti-inflammatory effects of IL-10 are mediated by STAT-3, which initiates signaling after phosphorylation and dimerization. For *in vitro* study, a chimeric STAT-3/estrogen receptor is activated upon exposure to the estrogen analog 4-hydroxytamoxifen (29). In contrast, targeted deletion of STAT-3 in macrophages leads to hyperresponsiveness to inflammatory stimuli such as LPS. Even in the absence of infection these mice develop a colitis similar to that observed in IL-10 knockout mice (30). In addition, disruption of STAT-3 leads to proliferation of neutrophils, demonstrating that STAT-3 impacts differentiation of the myeloid lineage (31).

We evaluated the anti-inflammatory role of IL-10 in macrophages. IL-10 induced inhibitory C/EBP β in macrophages, but deletion of the signaling molecule STAT-3 abrogated this effect.

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IL-10 was unable to induce inhibitory C/EBP β in monocytes, but maturation of monocytes to macrophages gained this ability, probably through induction of the post-translational effect of CUG-binding protein. These findings provide a basis of the anti-inflammatory arm of the host response to *Mycobacterium tuberculosis* after therapy is initiated.

MATERIALS AND METHODS

Lipoarabinomannan Stimulation of Monocyte-Derived Macrophages

Peripheral blood mononuclear cells (PBMC) were obtained from blood by Ficoll-Hypaque sedimentation, and buffy coats were washed four times in PBS. PBMC were suspended in RPMI 1640 with 10% FCS and allowed to adhere to plastic plates overnight; cells were then incubated with LPS (2 μ g/ml) or lipoarabinomannan (LAM; 1 μ g/ml). LPS-free Man-LAM was obtained from John Belisle (Colorado State University, NIAID, NIH Contract NO1-AI-75320). Supernatants were sampled at Day 1 and at 2-d intervals thereafter. ELISA (R&D Systems, Minneapolis, MN) were used to assay IL-10 concentrations.

Cell Culture

Murine bone marrow-derived macrophages were prepared as previously described (31). Briefly, tibial/fibial bone marrow from CO₂-killed mice were suspended in DMEM +10% FCS + 20% LCCM growth medium. Adherent cells were harvested by gentle scraping after 7 d, washed, and resuspended in RPMI at a density of 10⁶ cells/ml. The cells were treated with 2 μ g/ml and harvested 24 h after treatment. THP-1 and HL-60 cells were cultured in RPMI 1640 with 10% FCS. Cells were differentiated with 20 ng/ml phorbol 12-myristate 13-acetate (PMA) for 24 h or as indicated in each experiment. For experiments to examine the effect of IFN or IL-10 on C/EBP β , cells were either left undifferentiated or differentiated with PMA for 24 h and then treated with IFN- β or IL-10 as indicated. Peritoneal macrophages were isolated from STAT3 knockout mice or WT controls 3 d after injection with 3 ml of 3% brewers thioglycolate by peritoneal cavity lavage with 10 cc of PBS after mice were killed. Cells were suspended in RPMI containing 10% FCS + fungizone + penicillin/streptomycin at a density of 10⁶ cells/ml and plated in 6-well plates with 10⁶ cells/well.

HIV-1 Promoter Assay

For the model of activation of latent HIV-1 from monocytes or macrophages, we used BF 24 cells (AIDS Research and Reference Reagent Program #1296). Protein extracts for chloramphenicol acetyl transferase (CAT) ELISA (Roche Molecular Biochemicals, Mannheim, Germany) were processed according to the manufacturer's instructions. For pseudotyped virus infection experiments, the virus contained the envelope of HIV-1_{JR-FL} and the luciferase reporter gene replacing the envelope gene. Infection were performed as reported with an MOI of 0.5. Protein extracts for luciferase (Roche Molecular Biochemicals) were processed according to manufacturer's instructions (13).

Transfection

Transfections were done using a Gene Gun (Biorad) with 0.5 μ g of each plasmid (for 1 \times 10⁷ cells) mixed with 0.5 mg of gold. Transfections were performed using 50 and 100 psi to drive the gun using STAT-3-ER (29) and cotransfected with C/EBP β Luc (32). 4HT (1 μ mol/ml) was added for 24 h before cells were harvested; values were normalized to protein concentration.

Whole-Cell and Nuclear Extract Preparation and Immunoblot

Cells were washed twice in PBS. Whole-cell extracts were prepared for immunoblot analysis by incubation in RIPA buffer (PBS, 1% NP40, 0.5% deoxycholate) containing 3 μ g/ml aprotinin, 1 mM PMSF, and 1 mM Na orthovanadate for 30 min and disruption by passage through a 21-g needle. Proteins were separated by SDS-PAGE. For each gel, equal amounts of protein were loaded in each lane. From gel to gel, the amount of protein per lane was 30–100 μ g for detection of endogenous proteins. For detection of C/EBP β 10–20% linear-gradient gels were used. For detection of STAT-3, 10% gels were used. After electrophore-

sis, protein was electrotransferred to nylon membranes for detection of C/EBP β or STAT-3. Membranes were blocked with nonfat dry milk, then probed with antibodies against C/EBP β , STAT-3, or CUG-binding protein from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), visualized with anti-rabbit HRP and ECL (Amersham Inc., Arlington Heights, IL). Multiple film exposures were scanned by laser densitometry and analyzed with Imagequant software (Molecular Dynamics; Amersham Biosciences, Piscataway, NJ) for quantitation of immunoreactive STAT-3. Both the anti-C/EBP β and anti-STAT-3 antisera reacted with nonspecific bands, which confirmed equal protein loading from lane to lane.

Determination of Transcription Rates

Cells were treated as described. Run-on assays were performed as previously described (19). Radiolabeled nascent RNA was isolated and hybridized to excess plasmid DNA fixed to nitrocellulose. A β -actin clone was used as an internal standard. Bluescript KS- (Stratagene, La Jolla, CA) and RCMV (Invitrogen, Carlsbad, CA) were used as negative controls and to determine background signal. The results were visualized and quantitated with a phosphorimager and Imagequant software (Molecular Dynamics).

RESULTS

IFN and IL-10 Are Members of a Redundant Cytokine Network that Induces a C/EBP β Repressor in Macrophages but Not Monocytes

We have previously shown that production of the inhibitory transcription factor C/EBP β is an IFN effect that represses HIV-1 replication in macrophages after an inflammatory stimulus (13). Macrophages with targeted deletion of STAT-1 and the type I IFN receptor were used to define the role of IFN signaling in production of the inhibitory C/EBP β . Similar to human macrophages, wild-type murine bone marrow-derived macrophages stimulated with LPS upregulated both long-form stimulatory and short-form inhibitory C/EBP β (Figure 1, lane 2). Therefore,

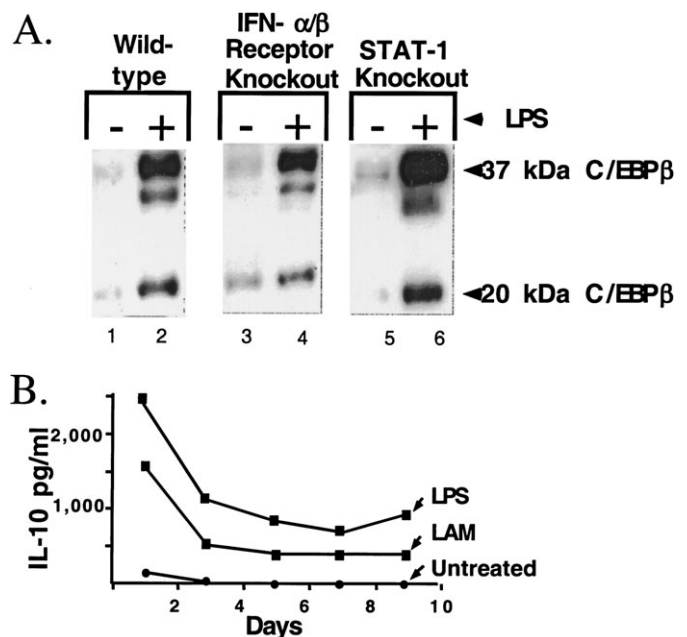


Figure 1. The effect of stimulation with LPS or infection with *M. tuberculosis* on C/EBP β expression and IL-10 production in macrophages. (A) C/EBP β expression in bone-marrow-derived macrophages from wild-type and mutant mice. The mutations in the IFN pathways are labeled above the immunoblot. Lanes 1, 3, and 5 are without LPS stimulation. Lanes 2, 4, and 6 are with LPS stimulation for 72 h. (B) Monocyte-derived macrophages were treated with LPS or *M. tuberculosis* LAM. The increase IL-10 was sustained over nine days of cell culture. LPS induced 2-fold more IL-10 than did infection with *M. tuberculosis*.

bone marrow-derived macrophages from mice are a good model to study regulation of inhibitory C/EBP β .

The C/EBP β repressor is induced by LPS in macrophages from mice deficient in the p100 type-I IFN (Figure 1, lanes 3 and 4) and in macrophages deficient in STAT-1 (Figure 1, lanes 5 and 6). IFN signaling is therefore not necessary for expression of the C/EBP β repressor in macrophages. This suggests that other cytokines released in response to LPS will also induce the repressor. Since IL-10 is a well-characterized anti-inflammatory cytokine that is induced by LPS or *M. tuberculosis* LAM, we measured IL-10 production in our system. Stimulation with LPS or LAM strongly induced IL-10. Of note high levels of IL-10 were maintained for 9 d in cell culture (Figure 1B). LPS induced higher levels of IL-10 than did LAM, and so subsequent experiments were conducted with LPS stimulation.

IL-10 Produces Inhibitory C/EBP β in Macrophages but Not Monocytes

HL-60 cells, a transformed monocytic cell line, were treated with IL-10 or IFN- β . IFN- β did not alter C/EBP β expression in monocytes but induced the inhibitory C/EBP β after differentiation to macrophages (Figure 2A, lanes 1 and 2). IL-10 ranging in concentration from 1–100 pg/ml does not alter C/EBP β expression in HL-60 monocytes (Figure 2A, lanes 3–5), but induced inhibitory C/EBP β in macrophages (lanes 6–8). Of note, IFN- β induced the inhibitory C/EBP isoform more strongly than did IL-10. Similar results were obtained with THP-1 and U937 cells demonstrating the effect of differentiation was not cell line specific (data not shown).

In the absence of cytokine treatment, there is little C/EBP β protein expression. Treatment of differentiated THP-1 macrophages with 100 pg/ml of IL-10 led to significant induction of inhibitory C/EBP β in 24 h (Figure 2B, lane 2). There was continued induction of C/EBP β up to 72 h after stimulation (lanes 3 and 4). Taken together, these results demonstrate that only differentiated macrophages produce inhibitory C/EBP β in response to cytokine stimulation.

A C/EBP β promoter reporter construct was used to test the transcriptional effects of IL-10 stimulation and STAT-3 activation on C/EBP β transcription. IL-10 increased C/EBP β promoter activity 3-fold, while IFN- β produced a 7-fold increase in

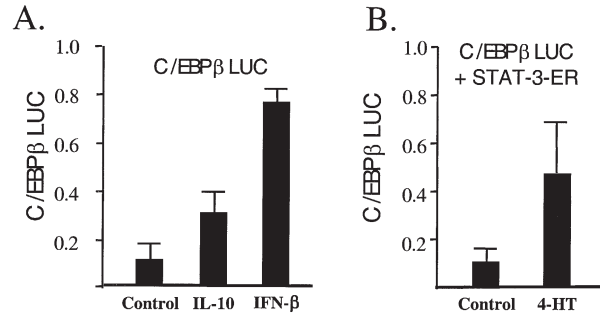


Figure 3. Stimulation of C/EBP β . (A) IFN- β or IL-10 stimulate C/EBP β promoter to produce luciferase in differentiated macrophages. IFN- β is more effective than IL-10. (B) Transfection of STAT-3/estrogen receptor (ER) plasmid results in dimerization of STAT-3/ER fusion protein after treatment with 4-hydroxy-tamoxifen. This results in stimulation of the C/EBP β promoter in undifferentiated THP-1 cells.

macrophage-like cells (Figure 3A). To confirm that activated STAT-3 is capable of inducing C/EBP β transcription, we used the C/EBP β reporter construct co-transfected with an expression plasmid expressing STAT-3-ER. STAT-3-ER is a chimeric protein containing the estrogen binding domain of the estrogen receptor and the DNA binding domain of STAT-3. 4-hydroxy-tamoxifen activates the fusion protein leading to induction of promoters with STAT-3 binding sites. C/EBP β promoter activity increased 5-fold when undifferentiated monocytic cells containing the STAT-3-ER construct were treated with 4-hydroxy-tamoxifen (Figure 3B).

IL-10 Inhibits HIV-1 Promoter Function and Replication in Macrophages but Not Monocytes

HIV-1 LTR promoter activity was used to test the functional effect of stimulation with IL-10. Initial experiments used BF-24 cells, which are THP-1 cells stably transfected with a CAT construct under the control of the HIV-1 LTR. In the absence of PMA differentiation, IL-10 had no effect on LTR activity in monocytic BF-24 cells, whereas IFN- β produced a 40% reduction. After differentiation, IL-10 inhibited HIV-1 LTR activity by 65%,

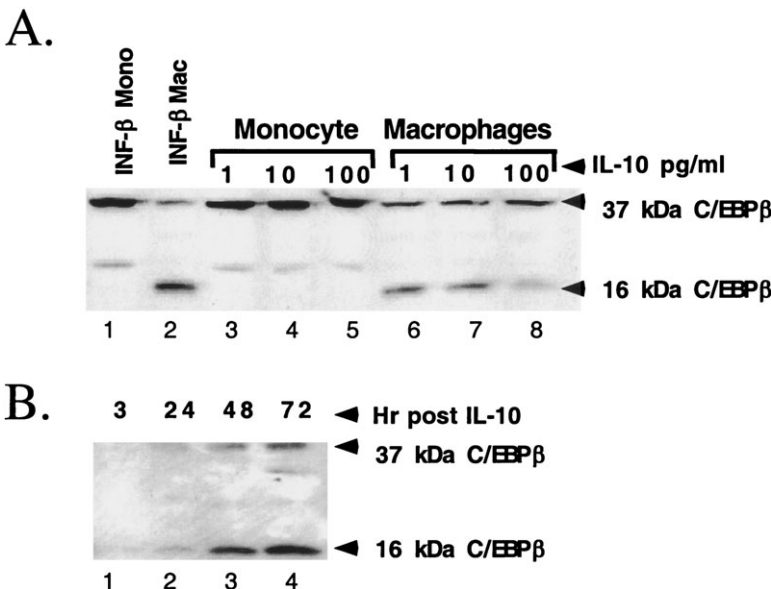


Figure 2. The effect of IL-10 on C/EBP β expression in monocytes and macrophages. (A) Dose response of undifferentiated and differentiated HL-60 cells. A Western blot demonstrates that macrophages produce inhibitory 16-kD C/EBP β after stimulation with either IFN- β or IL-10. Undifferentiated HL-60 monocytes fail to produce inhibitory 16-kD C/EBP β . (B) Time course of C/EBP β expression in differentiated THP-1 cells. There is significant induction of inhibitory C/EBP β 24 h after stimulation of THP-1 macrophages with 100 pg/ml of IL-10. The level of inhibitory C/EBP β continues to increase until 72 h after stimulation.

while IFN- β produced a 90% reduction in CAT reporter activity (Figure 4A).

A pseudotyped HIV-1, carrying HIV-1_{JR-FL} envelope with luciferase in place of the envelope gene, was used to confirm the conclusion of the LTR reporter construct. IL-10 did not alter HIV-1 LTR-driven luciferase reporter activity when pseudotyped HIV-1 infected undifferentiated THP-1 cells. IFN- β did produce a 40% reduction. Once THP-1 cells were differentiated by PMA into macrophages, both IL-10 and IFN- β produced a 50% decline in HIV-1 LTR luciferase reporter activity (Figure 4B). These results demonstrate that IL-10 has antiviral activity only in differentiated macrophage like cells.

STAT-3 Regulates C/EBP β Production after IL-10 Stimulation

Since STAT-3 is a transcription factor critical for IL-10 signaling, macrophages with targeted deletion of STAT-3 were used to test if STAT-3 was necessary for production of inhibitory C/EBP β after stimulation with IL-10 or LPS. Cultured wild-type mouse peritoneal macrophages do not express inhibitory C/EBP β (Figure 5A, lane 1). IL-10, LPS or a combination of these two stimuli induces the inhibitory isoform (Figure 5A, lanes 2–4). Peritoneal macrophages with targeted deletion of STAT-3 no longer produce the inhibitory isoform after stimulation with LPS or IL-10 (Figure 5A, lanes 6 and 7).

Differentiation Leads to Post-Transcriptional Upregulation of STAT-3 and Gain of IL-10 Response

To test if differentiation altered the level of STAT-3 protein, an immunoblot of extracts from monocytic and macrophage-like cells was performed. Differentiation produced a 3-fold increase in STAT-3 protein (Figure 6, compare lanes 1 and 2). To test if this was due to induction of STAT-3 transcription, a nuclear run was performed. Differentiation did not alter STAT-3 transcription (Figure 7). Transcription of osteopontin was induced over 50-fold, demonstrating that differentiation did alter transcription of some genes (Figure 7). The discrepancy between transcription and protein suggests that differentiation leads to post-transcriptional induction of STAT-3.

CUG-binding protein leads to production of inhibitory C/EBP β during *in vitro* translation (16). Since only macrophages

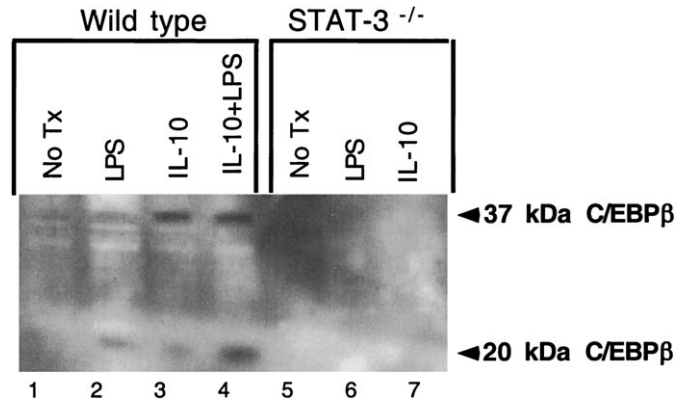


Figure 5. The effect of alteration of STAT-3 level and activity on C/EBP β expression in monocytes and macrophages. Wild-type peritoneal macrophages produce inhibitory C/EBP β after treatment with LPS or IL-10 (lanes 2 and 3). The combination is synergistic (lane 4). The STAT-3 knockout macrophages fail to induce C/EBP β after stimulation with both LPS and IL-10 (lanes 6 and 7).

produced inhibitory C/EBP β , nuclear extracts of monocytes and macrophages were tested for CUG-binding protein expression. In the absence of differentiation, no CUG-binding protein was detected (Figure 8, lane 1). After differentiation, macrophages strongly expressed CUG-binding protein (lane 2). There was further induction of CUG-binding protein six hours after treatment with IFN- β (compare lanes 3 and 4), while IL-10 treatment produced little induction of CUG-binding protein (compare lanes 5 and 6). The ability to produce inhibitory C/EBP β therefore correlates with the presence of CUG-binding protein.

DISCUSSION

We have demonstrated that induction of the inhibitory C/EBP β is an interferon effect in macrophages but not monocytes (19). Induction of this transcriptional repressor inhibits HIV-1 LTR promoter activity and viral replication (8, 11). When expression of the inhibitory C/EBP β is specifically inhibited by an antisense oligonucleotide, HIV-1 replication in macrophages is enhanced (33, 34). *In vivo* TB infection accelerates HIV-1 replication in alveolar macrophages through downregulation of inhibitory C/EBP β and activation of NF- κ B (3). We now report that like IFNs, the anti-inflammatory cytokine IL-10 induces the C/EBP β repressor in macrophages but not monocytes. Induction of C/EBP β by IL-10 may explain the inhibition of HIV-1 replication after PPD stimulation of CD8 cells *in vitro* (27). Further, IFN- γ , the type II IFN, is able to induce inhibitory C/EBP β in macrophages, and this may inhibit HIV-1 replication observed after

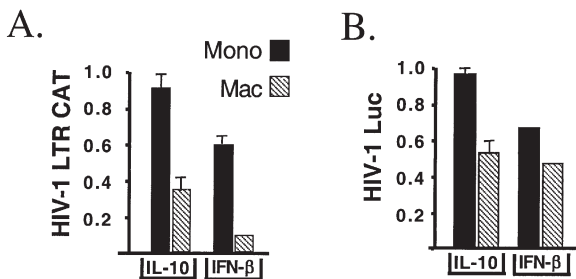


Figure 4. The effect of IL-10 on HIV-LTR activity and HIV-1 replication in monocytes and macrophages. (A) IL-10 has no effect on LTR activity in undifferentiated monocytic cells, while IFN- β produces a 40% reduction of LTR activity (black bars). IFN- β or IL-10 inhibit HIV-1 LTR-driven CAT production in differentiated BF-24 cells 24 h after treatment (hatched bars). IL-10 produces a 60% inhibition, while IFN- β produces a 90% inhibition. The reporter activity in the absence of stimuli is defined as 1. (B) IL-10 has no effect on mRNA expression after infection of monocytic cells with pseudotyped HIV-1 with luciferase substituted for the envelope gene. IFN- β produces a 40% reduction in undifferentiated cells. IL-10 and IFN- β produce a 50% reduction in reporter expression in differentiated THP-1 cells. The reporter activity in the absence of stimuli is defined as 1.

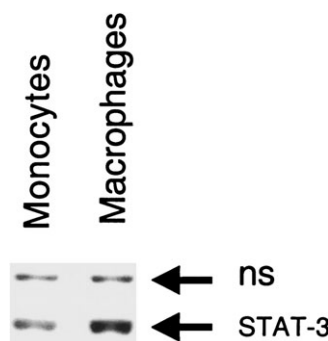


Figure 6. The effect of differentiation on STAT-3 protein. Differentiation of THP-1 monocytes to macrophages increased STAT-3 protein expression 3-fold. NS represents a nonspecific band used as an internal control for loading.

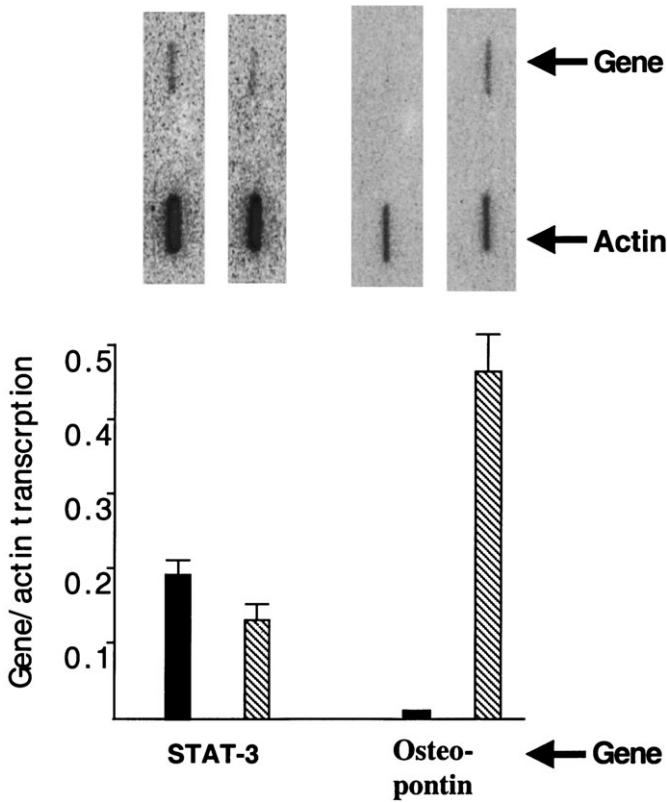


Figure 7. The effect of differentiation on STAT-3 transcription. Differentiation of THP-1 monocytes to macrophages does not increase transcription of STAT-3. Differentiation led to over a 50-fold increase in osteopontin transcription. Blot of run-on experiment presented at the top and quantitation presented at the bottom of the figure.

aerosolized IFN- γ (35). The state of differentiation appears to be the critical parameter controlling the ability of cells to produce this repressor in response to cytokine stimulation.

We used transformed monocytic cell lines THP-1 U937 and HL-60 to model the regulation of C/EBP β in monocytes. After treatment with PMA, these cells differentiate and regulate the C/EBP β and NF- κ B transcription factor expression like primary monocytes and macrophages (3, 13, 19). Undifferentiated THP-1 monocytes are unresponsive to IL-10. This cytokine does not

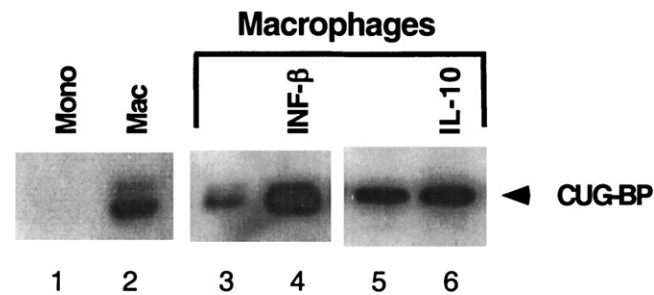


Figure 8. The effect of differentiation, IFN- β , and IL-10 on CUG-binding protein. Undifferentiated THP-1 cells do not express CUG-binding protein (lane 1). PMA differentiation strongly induces CUG-binding protein (lane 2). Six hours after treatment with 1 U/ml of IFN- β , there is strong induction of CUG-binding protein (compare lane 3 to lane 4). IL-10 treatment did not produce significant alteration of CUG-binding protein in macrophages (lanes 5 and 6).

affect C/EBP β expression or expression of transcription factors mediating IFN effect in undifferentiated cells. After differentiation, IL-10 induces transcription of STAT-3 in a positive autoregulatory loop and also increases STAT-3 protein. Evidence that supports this conclusion includes: (1) differentiated THP-1 cells express 3-fold higher STAT-3 protein concentration than do undifferentiated THP-1 cells, (2) murine macrophages deficient in STAT-3 are unable to induce the C/EBP β repressor after stimulation with IL-10 or LPS, and (3) engineered activation of a chimeric STAT-3-ER protein leads to enhanced C/EBP β promoter activity in undifferentiated THP-1 cells. These data demonstrate that activated STAT-3 is both necessary and sufficient for induction of C/EBP β after stimulation with IL-10.

Similar to the type I interferon system, the effect of differentiation is mediated by post-transcriptional induction of a transcription factor critical for cytokine signaling. In the case of IL-10, the signaling intermediate is STAT-3. In the case of type I interferon, the signaling intermediate is IRF-9. Since signaling by IFN- γ does not require IRF-9, monocytes are capable of responding to this cytokine. The effectiveness of the STAT-3-ER fusion in driving C/EBP β transcription in undifferentiated THP-1 cells demonstrates that other components of the transcriptional machinery required for expression of C/EBP β are present in monocytic cells. The CUG-binding protein was induced by differentiation. The presence of this RNA-binding protein led to production of inhibitory C/EBP β during *in vitro* translation (16). It is likely that upregulation of this protein during differentiation contributes to the ability of differentiated cells to produce the inhibitory C/EBP β in response to IL-10 and perhaps other cytokines.

The data reported in this investigation emphasize the importance of the state of differentiation in determining the response to cytokine stimulation. Differentiation induces latent transcription factors required for type I IFN and IL-10 responsiveness. This increased signaling capacity is complemented by a gain in the capacity to produce a C/EBP β repressor protein from an intronless mRNA. Formation of STAT-3 dimer or ISGF-3 would increase transcription of the C/EBP β gene. In monocytes, translation of this mRNA produces a stimulatory C/EBP β isoform and activate transcription of proinflammatory genes (19).

In macrophages, induction of C/EBP β transcription would lead to translation of a repressor, inhibiting transcription of the same proinflammatory genes that were stimulated in monocytes (9, 10). Repression of cytokine genes that contain C/EBP sites may explain the deleterious effect of high levels of IL-10 production on the inflammatory response during tuberculosis (23–27).

In active inflammation in the lower respiratory tract in TB, sarcoidosis, asbestosis, etc., there is recruitment of monocytes, which can produce cytokines that amplify inflammation. As the inflammation declines, IL-10 and differentiated macrophages can further dampen inflammation by induction of inhibitory C/EBP β protein. Also, since C/EBP sites exist in the promoters of many viral genes (36), expression of the C/EBP β repressor in resting alveolar macrophages will affect many viral types and may be one mechanism of resistance to viral infection of the lung.

It is likely that the alterations in RNA processing and translation induced by differentiation will affect other pathways. Expression of the repressor is abolished when activated lymphocytes contact the macrophage (3). This contact-mediated derepression then produces a macrophage capable of producing proinflammatory cytokines, supporting high-level HIV-1 replication. The interplay of repression and derepression will influence the large number of inflammatory genes containing promoters with C/EBP sites. This interplay will likely be pivotal in determining if a given stimulus leads to up- or downregulation of inflammation and HIV-1 replication.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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