Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11518–11522, December 1992
Cell Biology

Tumor necrosis factor α response elements in the *HLA-DRA* promoter: Identification of a tumor necrosis factor α-induced DNA–protein complex in astrocytes

R. BRIAN PANEK*, HAROLD MOSES†, JENNY P.-Y. TING†‡§, AND ETTY N. BENVENISTE*¶

*Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294; and †Curriculum in Neurobiology, ‡Lineberger Cancer Research Center, and §Department of Microbiology-Immunology, University of North Carolina, Chapel Hill, NC 27599

Communicated by Dominick P. Purpura, June 25, 1992

ABSTRACT **The cytokine tumor necrosis factor α (TNF-α) alone does not induce class II major histocompatibility complex (MHC) expression in most primary cells but can regulate ongoing class II expression in either a positive or negative fashion. The mechanism(s) by which TNF-α enhances interferon γ (IFN-γ)-induced class II expression was examined in a primary cell type, the astrocyte, by transient transfection of the *HLA-DRA* promoter linked to a chloramphenicol acetyltransferase reporter gene (DRA-CAT). We show that TNF-α, while having no effect on its own, can synergize with IFN-γ to increase the level of promoter activity of a DRA-CAT construct. Three known sequences—W, X, and Y—are required for TNF-α enhancement of IFN-γ-induced promoter activity. The corollary effect of TNF-α on DNA-binding proteins specific for these elements was examined. A previous report described a DNA-binding protein, IFN-γ-enhanced factor X (IFNEX), which is upregulated by IFN-γ in astrocytes and is specific for the X box of the *DRA* promoter. In this study, we found that TNF-α alone did not induce any nuclear proteins; however, combined treatment of astrocytes with both IFN-γ and TNF-α induced a DNA–protein complex of slower electrophoretic mobility than IFNEX. The TNF-α-induced complex (TIC-X) has specificity for the X element of the *DRA* promoter. These results suggest a mechanism by which TNF-α enhances IFN-γ-induced class II MHC expression via the formation of TIC-X.**

Class II antigens are heterodimeric glycoproteins encoded by genes in the major histocompatibility complex (MHC) which play a critical role in the induction of immune responses (1). The constitutive expression of class II MHC antigens is restricted to B cells, dendritic cells, thymic epithelial cells, and macrophages (2), although other cell types can express class II antigens upon exposure to interferon γ (IFN-γ). These include fibroblasts (3), pancreatic β cells (4), endothelial cells (5), and astrocytes (6). The proper constitutive and inducible expression of class II antigens is a crucial determinant in the generation of normal immune responses, and aberrant class II expression has been implicated in various autoimmune disorders (7).

IFN-γ-induced class II gene expression is for the most part transcriptionally regulated. Three cis-acting elements which are highly conserved between murine and human class II MHC promoters have been described; from 5′ to 3′ they are the W (Z, S, or H), X, and Y elements, respectively, which are important for both constitutive and IFN-γ-induced class II expression (for reviews see refs. 8 and 9).

Although IFN-γ is considered the primary inducer of class II antigens, tumor necrosis factor α (TNF-α) can both enhance and inhibit IFN-γ-induced class II expression (10–12). TNF-α is usually unable to initiate transcription of class II genes but can either positively or negatively regulate ongoing class II expression. Few studies have been performed to understand the mechanism(s) by which TNF-α enhances IFN-γ-induced class II expression, particularly in primary cells. This report utilizes primary rat astrocytes for this reason. Class II MHC expression in the central nervous system is normally absent, but induced expression on astrocytes has been observed in various neurological diseases (for review see ref. 13).

Previous work has shown that astrocytes express class II MHC mRNA and protein in response to IFN-γ, that TNF-α alone has no effect on class II expression, and that TNF-α enhances IFN-γ-induced class II expression (10, 14). TNF-α acts at the transcriptional level to increase IFN-γ-induced transcription of the class II gene as assessed by nuclear run-on assays and does not act posttranscriptionally to stabilize IFN-γ-induced class II mRNA (14). We recently demonstrated that elements within the W, X, and Y sequences are essential for IFN-γ inducibility of the *HLA-DRA* gene in astrocytes, and we described a DNA-binding protein, IFN-γ-enhanced factor X (IFNEX), which is upregulated by IFN-γ and is specific for the X box (15, 16). We have extended these studies to examine the molecular mechanism(s) by which TNF-α enhances IFN-γ-induced class II gene expression. We show that the W, X, and Y sequences are critical for TNF-α enhancement of IFN-γ-induced *DRA* gene expression in astrocytes. More importantly, we describe a DNA–protein complex [TNF-α-induced complex X (TIC-X)] that is induced by a combined treatment of astrocytes with IFN-γ and TNF-α and is specific for the X element of the *DRA* promoter.

MATERIALS AND METHODS

Recombinant Proteins. Recombinant rat IFN-γ was purchased from AMGen Biologicals, and recombinant human TNF-α was the generous gift of Genentech.

Primary Glial Cell Cultures. Primary glial cell cultures were established from neonatal rat cerebra (17). Astrocyte cultures were routinely >97% positive for glial fibrillary acidic protein, an intracellular antigen unique to astrocytes (18).

Transfection and Chloramphenicol Acetyltransferase (CAT) Assay. *HLA-DRA*/CAT gene constructs used in these studies have been described in detail previously (15, 16) and are shown schematically in Fig. 1. Transfection of primary rat astrocytes was performed by electroporation (16) with 15 μg of plasmid DNA per transfection. Transfected astrocytes

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CAT, chloramphenicol acetyltransferase; hXBP-1, human X-box-binding protein 1; IFN-γ, interferon γ; IFNEX, IFN-γ-enhanced factor X; MHC, major histocompatibility complex; TNF-α, tumor necrosis factor α; TIC-X, TNF-α-induced complex X; TIP, TNF-α-induced protein.

¶To whom reprint requests should be addressed.

were either unstimulated or stimulated for 48 hr with IFN-γ (100 units/ml), TNF-α (50 ng/ml), or both and then harvested, and protein extracts were prepared (19). CAT activity was determined by the liquid scintillation counting method of Seed and Sheen (20). Fold induction of CAT activity after IFN-γ or IFN-γ/TNF-α treatment was calculated by dividing the cpm of the IFN-γ- or IFN-γ/TNF-α-treated sample by the cpm of the untreated sample.

Nuclear Extracts and DNA Binding Assays. Nuclear extracts were prepared (16) from astrocytes which were incubated without or with IFN-γ (100 units/ml), TNF-α (50 ng/ml), or both for 24 hr. Gel shift assays were performed (16), using as probes (*i*) a DNA fragment extending from base pair (bp) −152 to bp −66 isolated from Δ−152-WT, (*ii*) X+Y oligonucleotide (−110 to −61, 5′-CCCCTAGCAACAGATGCGTCATCTCAAAATATTTTTCTGATTGGCCAAAG-3′), and MX+Y oligonucleotide (−110 to −61, 5′-CC**AAGCTACCACTCGTA**GTCATCTCAAAATATTTTTCTGATTGGCCAAAG-3′). For competition reactions, nonradioactive competitor was incubated with the nuclear extract for 5 min prior to the addition of labeled probe. Reaction mixtures were then electrophoresed at 200 V in 6% polyacrylamide gels in 1× TBE (89 mM Tris base/89 mM boric acid/2.5 mM EDTA, pH 8.3). The dried gels were exposed to Kodak XAR-5 film at −70°C with intensifying screens for various times.

RESULTS

Deletion and Substitution Mutants Demonstrate That the W, X, and Y Elements Are Required for TNF-α Enhancement of IFN-γ-Induced *DRA* Expression. Previous work has shown that a 141-bp region of the *DRA* promoter, including the W, X, and Y boxes, is required for IFN-γ induction of class II expression in astrocytes (15). To determine which region(s) of the *DRA* promoter is needed for the enhancing activity of TNF-α, we used constructs that contained varying lengths of the 5′ end of the *DRA* promoter (Fig. 1*A*). These constructs were transiently transfected into primary rat astrocytes and tested for their response to IFN-γ, TNF-α, or IFN-γ plus TNF-α. In agreement with our previous findings (15), IFN-γ inducibility was maintained down to −141 bp but was lost at −109 bp (Fig. 1*A*). TNF-α enhancement of IFN-γ-induced DRA promoter activity exhibited a similar pattern. Cells treated with IFN-γ/TNF-α after transfection with constructs 5′Δ−1028 and 5′Δ−141 showed a consistent increase in promoter activity compared with IFN-γ alone, whereas astrocytes transfected with construct 5′Δ−109 showed no response to IFN-γ and no enhancement in the presence of TNF-α (Fig. 1*A*). TNF-α alone had no effect on *DRA* promoter activity, giving essentially the same activity as unstimulated controls (data not shown). To define in more detail regulatory sequence(s) within 141 bp of the *DRA* promoter that contribute to TNF-α enhancement of IFN-γ-induced class II expression, we used a set of substitution mutants that individually affected the W, X, or Y region. Within the W region there is an authentic Servenius septamer (S2) (21) flanked by two potential Servenius-like sequences (S1 and S3) (see Fig. 1*B*). Mutation of the authentic Servenius sequence (W-M2) resulted in a substantial decrease in IFN-γ inducibility when compared with wild type (Fig. 1*B*), and also diminished, but did not abolish, the TNF-α effect. Mutation of either of the lesser conserved septamers (W-M1 and W-M3) had no effect on either the IFN-γ or the IFN-γ/TNF-α response. Mutations within the Y box that disrupted the inverse CCAAT motif (Y-M1) completely abolished IFN-γ inducibility and TNF-α enhancement (Fig. 1*B*).

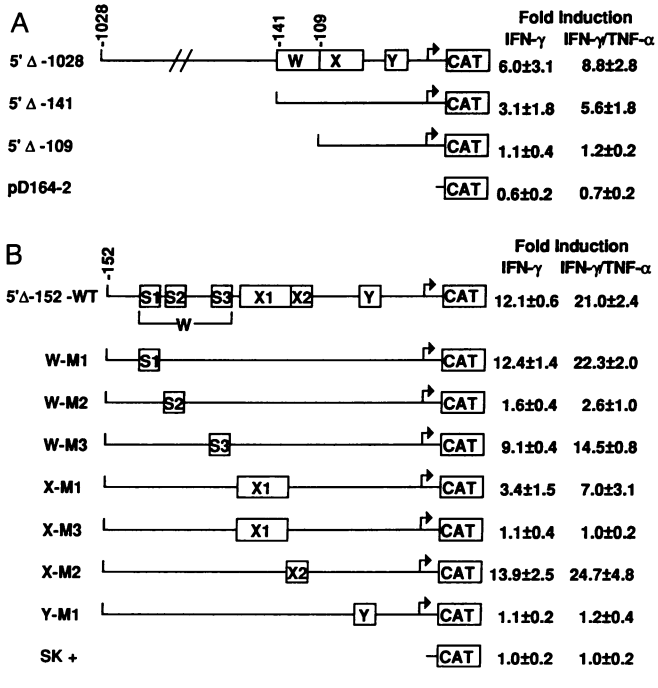

FIG. 1. Effect of deletion mutations (*A*) or site-specific mutations (*B*) in the *HLA-DRA* promoter on inducibility by IFN-γ or IFN-γ/TNF-α. Constructs were transiently transfected into astrocytes, and the cells were treated with medium alone, IFN-γ (100 units/ml), TNF-α (50 ng/ml), or IFN-γ plus TNF-α for 48 hr. Data are the means ± SD from 14 experiments (*A*) or five experiments (*B*). pD164-2 and SK+ serve as negative controls; the *DRA* promoter has been excised in these plasmids, which contain only the CAT gene.

Within the X region of the *DRA* promoter, the 5′ X_1 site is bound by the nuclear protein RF-X (22), while the 3′ X_2 site is bound by human X-box-binding protein 1 (hXBP-1) (23). The wild-type X_1 site has the sequence 5′-CCCCTAGCAACAGA-3′. A complete mutation of the X_1 site (X-M3; 5′-**AGAACTAGTCCAGA**-3′) abolished the response to both IFN-γ and IFN-γ/TNF-α (Fig. 1*B*). Mutation of the X_2 site (X-M2), which prevents binding of hXBP-1, had no effect on either induction by IFN-γ or enhancement by TNF-α. These results demonstrate that sequences within the X_1 but not X_2 site are required for both IFN-γ induction and TNF-α enhancement of *DRA* promoter activity in astrocytes.

IFN-γ/TNF-α Induces a Complex That Binds Specifically to the X Region of the *DRA* Promoter. We next examined whether TNF-α induces proteins which bind to the DRA promoter. Gel shift assays were performed using an oligonucleotide that contained the X+Y sequence (see Fig. 2*A* for a description of fragments and oligonucleotides used in the gel shift assays). The probe was radiolabeled and incubated with nuclear extracts from astrocytes that had been incubated with medium alone (untreated), IFN-γ, TNF-α, or IFN-γ/TNF-α for 24 hr. A constitutive complex was detected in extracts from untreated astrocytes (Fig. 2*B*, lane 1), and IFN-γ enhanced this complex (lane 2, complex 1, IFNEX), as previously observed (16). IFN-γ/TNF-α treatment induced the formation of a complex with slower mobility than IFNEX (lane 4, complex 2), whereas treatment with TNF-α alone was comparable to no treatment (lane 3). Competition with oligonucleotides corresponding to various regions of the *DRA* promoter demonstrated that only X+S competed for complex 1 and 2 formation (Fig. 2*C*, lanes 8 and 13). These results indicate that IFN-γ/TNF-α induces a nuclear protein(s) with specificity for the X box of the *DRA* promoter. We have tentatively named complex 2 as TIC-X. To better define the importance of the X region for complex formation, gel shift assays were performed using an oligonucleotide containing a mutated X region. Formation of TIC-X was evident upon incubation with the X+Y probe (Fig. 2*D*, lane 4) and was blocked by homologous oligonucleotide compet-

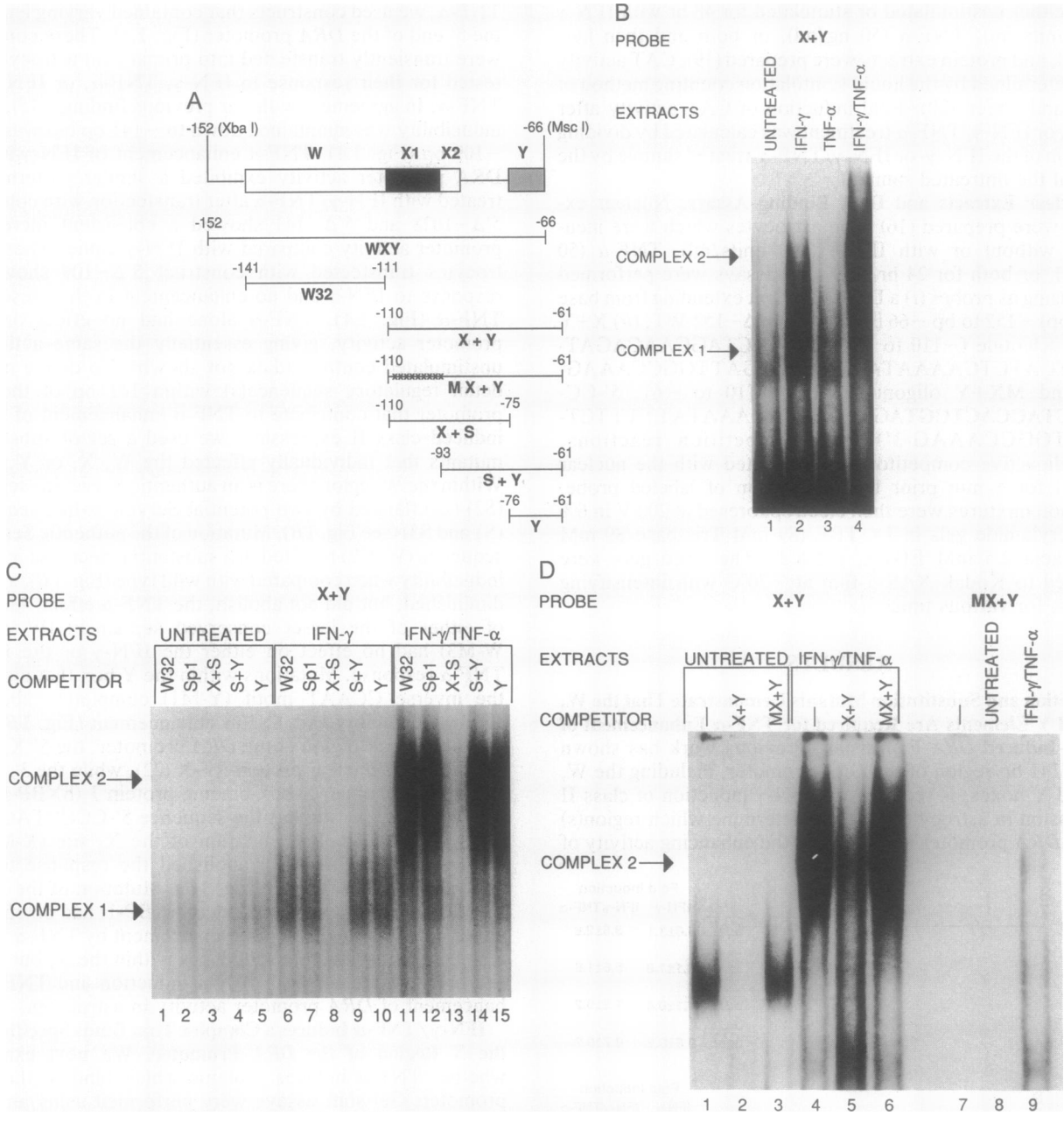

FIG. 2. (*A*) Restriction fragment and oligonucleotides used as probes and/or competitors in gel shift assays. The WXY probe was cut out of Δ−152-WT by using *Xba* I and *Msc* I (−152 to −66). All the other probes were synthetic oligonucleotides. Numbers refer to nucleotide positions relative to the *DRA* cap site. (*B*) IFN-γ/TNF-α treatment induces a protein(s) which complexes with the X+Y sequence of the *DRA* promoter. The X+Y probe (50,000 cpm per lane) was incubated with 10 μg of the indicated nuclear extract. The gel was exposed to film for 16 hr. (*C*) Competition experiment. A 100-fold molar excess of the indicated oligonucleotides was incubated with 10 μg of the indicated nuclear extracts for 5 min before the addition of 50,000 cpm of X+Y probe. The gel was exposed to film for 18 hr. (*D*) Mutation of the X box abolishes TIC-X formation. About 40,000 cpm of either the X+Y oligonucleotide (lanes 1–6) or the MX+Y oligonucleotide (lanes 7–9) was incubated without (lane 7) or with 10 μg of nuclear extract from untreated (lanes 1–3, and 8) or IFN-γ/TNF-α-treated (lanes 4–6, and 9) primary astrocytes, in the presence or absence of a 100-fold molar excess of the indicated competitor. The gel was exposed to film for 17 hr.

itor (lane 5), but not by mutated X-region oligonucleotide MX+Y (lane 6). In addition, TIC-X formation was not seen when the MX+Y oligonucleotide was used as probe (lane 9). These data indicate that the X region is critical for TIC-X formation. In contrast to our previous experience with gel shift assays which resulted in tight bands (24–26), the complexes reported in this manuscript and by Moses *et al.* (ref. 16, which describes IFNEX in detail) consistently formed a diffuse and broad band. We believe this pattern of complex formation is due to the nature of the nuclear proteins under study and their interactions with the *DRA* promoter.

Due to the inherent variability in working with primary cells, the reproducibility of these findings is critical. Thus, 65 gel shift assays have been performed with nuclear extracts from 15 different preparations of astrocytes, using multiple preparations of eight different DNA probes. We have consistently observed the formation of IFNEX and TIC-X.

Kinetic Analysis of IFNEX and TIC-X Formation. We next examined the time course of formation of IFNEX and TIC-X. Astrocytes were exposed to IFN-γ or IFN-γ/TNF-α for 6, 12, or 24 hr, and nuclear extracts were prepared and analyzed for binding to the −152 to −66 probe (WXY; see Fig. 2*A*). IFNEX was not detected until after a 12-hr stimulation with IFN-γ (Fig. 3*A*, lane 2) and persisted for at least 24 hr (lane 3). IFNEX was detected after only a 6-hr exposure to IFN-γ/TNF-α (lane 4); however, TIC-X was not observed

after 6 hr. After a 12-hr stimulation, IFNEX was further enhanced and TIC-X was just barely detectable (lane 5). Interestingly, after a 24-hr exposure to IFN-γ/TNF-α, the TIC-X complex was quite evident whereas IFNEX had decreased (lane 6). These results suggest that the combined treatment of astrocytes with IFN-γ and TNF-α has several effects with respect to formation of DNA–protein complexes: (*i*) an increase in the amount of IFNEX complex compared with that seen after treatment with IFN-γ alone, (*ii*) a decrease in the length of exposure to IFN-γ (in the presence of TNF-α) required for IFNEX complex formation, and (*iii*) the priming of astrocytes by IFN-γ so that they respond to TNF-α stimulation by the formation of a protein(s) [TNF-α-induced protein (TIP)] that results in the TIC-X complex. To confirm that an IFN-γ priming signal is required for TIC-X formation, experiments were performed in which nuclear extracts from IFN-γ- *or* TNF-α-treated astrocytes were mixed and then analyzed for binding to the WXY probe. Mixing the two extracts did not lead to TIC-X complex formation (Fig. 3*B*, lane 3), suggesting that a precursor form of TIP was not present in the nuclear extracts from astrocytes treated with TNF-α *alone*.

DISCUSSION

In this report, we have analyzed the responsiveness of a MHC class II promoter, that of the *HLA-DRA* gene, to the combined stimuli of IFN-γ plus TNF-α in a primary cell type, the astrocyte. We show that TNF-α has no effect on its own but can synergize with IFN-γ to increase the promoter activity of a *DRA*/CAT construct. The use of substitution mutants indicates that three sequence elements (W, X, and Y) are required for TNF-α enhancement of IFN-γ-induced promoter activity. Most crucial, we have correlated the *in vivo* functional analysis of *DRA* regulatory elements with gel mobility-shift assays for IFN-γ/TNF-α-induced DNA-binding proteins. Specifically, we have identified the formation of a DNA–protein complex with nuclear extracts from astrocytes treated with both IFN-γ and TNF-α; this complex, TIC-X, is specific for the X region of the *DRA* promoter.

A study of TNF-α induction of the murine class II gene *Aa* has been performed in WEHI-3 cells that differs from our study in that TNF-α alone induced class II gene expression in this cell line. Linker-scanning mutation of *Aa* demonstrated that five promoter elements were needed for TNF-α induction in WEHI-3 cells (27). These included the three conserved elements (H, X, and Y), the NF-κB-like enhancer, and a TNF-α-specific sequence called the T box. The T box is located between bp −31 and −18 of *Aa*, downstream from the Y box. The T-box sequence is important for optimal induction by TNF-α, but not IFN-γ. However, the full T-box sequence is found only in the *Aa* and *DQA* promoters.

TNF-α induces several transcription factors, including AP-1 and NF-κB. In primary astrocytes, TNF-α induces expression of the interleukin 6 gene, which is accompanied by a rapid and transient increase in the appearance of an NF-κB-like protein (26). Indications that TIC-X is not related to NF-κB include (*i*) a functional NF-κB binding site is not observed in the region of the *DRA* promoter under study; (*ii*) the appearance of NF-κB is rapid (within 15 min and lasting for 1–2 hr), while that of TIC-X is slow (optimal at 24 hr); (*iii*) NF-κB is activated by TNF-α alone, whereas TIC-X expression requires both IFN-γ and TNF-α; and (*iv*) an oligonucleotide containing the NF-κB sequence does not compete for the formation of TIC-X (data not shown).

TNF-α causes the transcriptional activation of c-Jun and c-Fos, which together constitute the major components of AP-1 (28). A c-Jun-like protein which can form a heterodimer with c-Fos or a related protein has been identified and cloned (23, 29). This protein, hXBP-1, binds to the X_2 site of the *DRA* promoter, which is almost a perfect match to the phorbol ester response element consensus sequence (23). Our transfection studies show that mutations in the hXBP-1 binding site (X-M2) have no effect on the IFN-γ or IFN-γ/TNF-α response, thus negating the possible involvement of hXBP-1 in *DRA* regulation by IFN-γ/TNF-α in the astrocyte.

TIC-X formation is induced only by a combined treatment with IFN-γ plus TNF-α, and the appearance of TIC-X occurs in a delayed, time-dependent manner. These findings suggest that astrocytes require a priming signal initiated by IFN-γ,

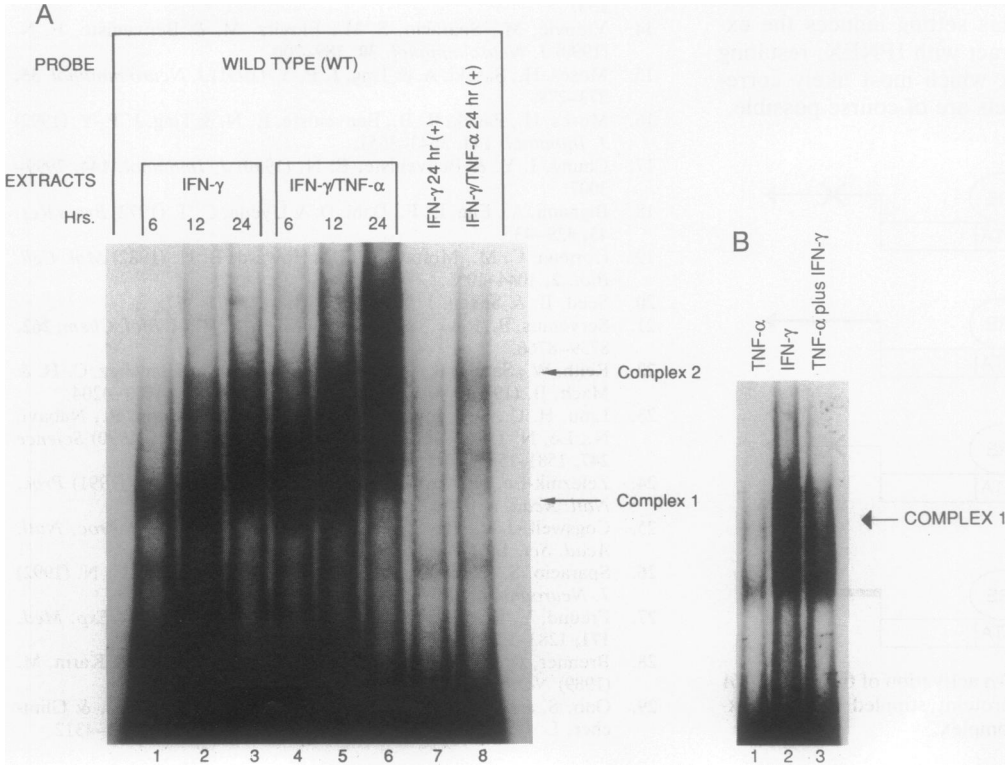

FIG. 3. (*A*) Kinetic analysis of the effect of IFN-γ or IFN-γ/TNF-α treatment on astrocyte formation of IFNEX and TIC-X. Nuclear extracts were prepared from primary astrocytes treated with either IFN-γ or IFN-γ/TNF-α for 6, 12, and 24 hr. The WXY (wild-type, WT) probe (60,000 cpm per lane) was incubated with 10 μg of nuclear extract. Lanes 1–3, IFN-γ-treated extract; lanes 4–6, IFN-γ/TNF-α-treated extract; lanes 7 and 8, IFN-γ- or IFN-γ/TNF-α-treated extracts plus a 100-fold molar excess of WT competitor DNA. The gel was exposed to film for 18 hr. (*B*) Gel shift analysis of nuclear extracts from IFN-γ- or TNF-α-treated astrocytes mixed together. The WXY (WT) probe (60,000 cpm per lane) was incubated with 10 μg of TNF-α-treated extract (lane 1), 10 μg of IFN-γ-treated extract (lane 2), or 5 μg of TNF-α-treated extract plus 5 μg of IFN-γ-treated extract (lane 3). The gel was exposed to film for 12 hr.

which then renders the astrocyte responsive to TNF-α, resulting in the subsequent formation of TIC-X. As shown by gel shift analysis, both IFNEX and TIC-X bind to the same region of the *DRA* promoter, and the appearance of TIC-X is *generally* accompanied by a decrease and/or disappearance in IFNEX. These data suggest that TIC-X may result from an interaction of DNA with IFNEX, and subsequently with TIP, leading to the appearance of the higher molecular weight complex, TIC-X. Preliminary UV crosslinking studies with a bromodeoxyuridine-substituted WXY probe indicate that there are no differences between extracts prepared from cells stimulated with IFN-γ or IFN-γ/TNF-α in either the number or the sizes of proteins which are *directly* interacting with the DNA. This suggests that TIP does not bind directly to DNA but in all likelihood binds to a DNA–IFNEX complex, and thus leads to TIC-X formation. These results do not exclude the possibility that TIC-X may actually be an IFNEX homomultimer. Both these interpretations are compatible with the data from transfection studies showing that as long as some responsiveness to IFN-γ is observed, TNF-α enhances promoter activity, but when the IFN-γ response is completely lost, there is no longer TNF-α enhancement. It is also possible, however, that IFNEX and TIP bind to different regions of DNA within the X box.

We propose a *tentative* model for TNF-α enhancement of IFN-γ induced class II MHC promoter activity and TIC-X formation (Fig. 4). Under basal conditions, astrocytes contain low levels of a constitutive protein which appears to bind to the X box; however, the class II promoter is transcriptionally inactive (a). An IFN-γ enhanced complex (IFNEX) is observed upon treatment with IFN-γ, which may represent an enhancement of either the binding activity or expression of the constitutive DNA-binding protein, resulting in transcriptional activation of the class II promoter (b). TNF-α treatment alone does not modify expression of the constitutive protein or activate transcription (c). Upon IFN-γ/TNF-α treatment, IFNEX is detected initially, and subsequently TIC-X is expressed. We hypothesize that IFN-γ, in addition to intensifying the IFNEX signal, also provides a priming signal to the astrocyte so that this cell is now responsive to TNF-α stimulation. TNF-α in this setting induces the expression of TIP, which may interact with IFNEX, resulting in a DNA–IFNEX–TIP complex which most likely corresponds to TIC-X (d). Other models are of course possible.

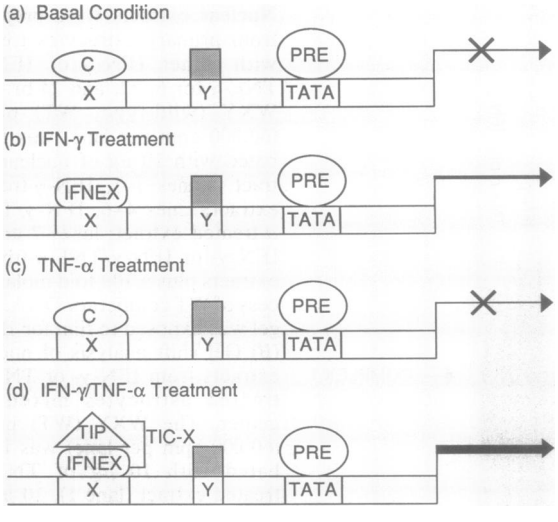

FIG. 4. Model for IFN-γ and TNF-α activation of the *HLA-DRA* promoter. C, constitutive nuclear protein; stippled box, Y-box-binding protein; PRE, preinitiation complex.

The requirement of three cis-acting elements (W, X, and Y) for IFN-γ- and IFN-γ/TNF-α-mediated class II MHC gene expression suggests that factors binding to these elements may interact with one another. Thus far, by gel shift analysis, we have not observed any complexes binding to the W box alone (data not shown); DNase footprinting analysis will determine whether there are factors other than the X-box-binding proteins present. A number of elements and transcription factors are likely to mediate the induction of class II expression following IFN-γ treatment, and its enhancement by TNF-α. IFNEX, TIP, and TIC-X may have a critical role in this process.

We thank Kathie Thomas for excellent secretarial assistance. This work was supported by Grants RG-2205-A-3 (E.N.B.), RG-2269-A-4 (E.N.B.), and RG-1785-B (J.P.-Y.T.) from the National Multiple Sclerosis Society; National Institutes of Health Grant AI27290 (E.N.B.); and National Cancer Institute Grant CA29589 (J.P.-Y.T.). H.M. was a National Institutes of Health Predoctoral Trainee (NS 07166), and R.B.P. is a current National Institutes of Health Predoctoral Trainee (AI07051).

1. Benacerraf, B. (1981) *Science* **212**, 1229–1238.
2. Hammerling, G. J., Mauve, G., Goldberg, E. & McDevitt, H. O. (1975) *Immunogenetics* **1**, 428–437.
3. Basham, B. Y. & Merigan, T. C. (1983) *J. Immunol.* **130**, 1492–1494.
4. Markmann, J., Lo, D., Naji, A., Palmiter, R. D., Brinster, R. L. & Heber-Katz, E. (1988) *Nature (London)* **336**, 476–481.
5. Pober, J. S., Gimbrone, M. A., Cotran, R. S., Reiss, C. S., Burakoff, S. J., Fiers, W., Rothlein, R. & Springer, T. A. (1983) *J. Exp. Med.* **157**, 1339–1353.
6. Fierz, W., Endler, B., Reske, K., Wekerle, H. & Fontana, A. (1985) *J. Immunol.* **134**, 3785–3793.
7. Massa, P. T., ter Meulen, V. & Fontana, A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4219–4223.
8. Benoist, C. & Mathis, D. (1990) *Annu. Rev. Immunol.* **8**, 681–715.
9. Cogswell, J. P., Zeleznik-Le, N. & Ting, J. P.-Y. (1991) *Crit. Rev. Immunol.* **11**, 87–112.
10. Benveniste, E. N., Sparacio, S. M. & Bethea, J. R. (1989) *J. Neuroimmunol.* **25**, 209–219.
11. Arenzana-Seisdedos, A., Mogensen, S. C., Vuillier, F., Fiers, W. & Virelizier, J. L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6087–6091.
12. Watanabe, Y. & Jacob, C. O. (1991) *J. Immunol.* **146**, 899–905.
13. Benveniste, E. N. (1992) *Chemical Immunology: Neuroimmunoendocrinology*, ed. Blalock, J. E. (Karger, Basel), Vol. 52, pp. 106–153.
14. Vidovic, M., Sparacio, S. M., Elovitz, M. & Benveniste, E. N. (1990) *J. Neuroimmunol.* **30**, 189–200.
15. Moses, H., Sasaki, A. & Ting, J. P.-Y. (1991) *J. Neuroimmunol.* **35**, 273–278.
16. Moses, H., Panek, R. B., Benveniste, E. N. & Ting, J. P.-Y. (1992) *J. Immunol.* **148**, 3643–3651.
17. Chung, I. Y. & Benveniste, E. N. (1990) *J. Immunol.* **144**, 2999–3007.
18. Bignami, A., Eng, L. F., Dahl, D. & Uyeda, C. T. (1972) *Brain Res.* **43**, 429–435.
19. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
20. Seed, B. & Sheen, J. Y. (1988) *Gene* **67**, 271–277.
21. Servenius, B., Rask, L. & Peterson, P. A. (1987) *J. Biol. Chem.* **262**, 8759–8766.
22. Reith, W., Satola, B. S., Kobr, M., Reinhart, D., Sanchez, C. H. & Mach, B. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4200–4204.
23. Liou, H. C., Boothby, M. R., Finn, P. W., Davidon, R., Nabavi, N., Le, N. J. Z., Ting, J. P.-Y. & Glimcher, L. H. (1990) *Science* **247**, 1581–1584.
24. Zeleznik-Le, N. J., Azizkhan, J. C. & Ting, J. P.-Y. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1873–1877.
25. Cogswell, J. P., Basta, P. V. & Ting, J. P.-Y. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7703–7707.
26. Sparacio, S. M., Zhang, Y., Vilcek, J. & Benveniste, E. N. (1992) *J. Neuroimmunol.* **39**, 231–242.
27. Freund, Y. R., Dedrick, R. L. & Jones, P. P. (1990) *J. Exp. Med.* **171**, 1283–1299.
28. Brenner, D. A., O'Hara, M., Angel, P., Chojkier, M. & Karin, M. (1989) *Nature (London)* **337**, 661–663.
29. Ono, S. J., Liou, H. C., Davidon, R., Strominger, J. L. & Glimcher, L. H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4309–4312.