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Anti-β-interferon antibodies inhibit the increased expression of HLA-B7 mRNA in tumor necrosis factor-treated human fibroblasts: Structural studies of the β_2 interferon involved

(autocrine interferons/cDNA cloning/nucleotide sequence analysis/Sellers TT algorithm)

LESTER T. MAY, DAVID C. HELFGOTT, AND PRAVINKUMAR B. SEHGAL

The Rockefeller University, New York, NY 10021

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ABSTRACT **Recombinant *Escherichia coli*-derived human tumor necrosis factor (TNF) induces the 1.3-kilobase β_2 interferon (IFN-β_2) mRNA in human diploid fibroblasts (FS-4 strain). IFN-β_2 is serologically related to the well-characterized IFN-β_1 (respective antisera cross-neutralize the heterologous protein). Polyclonal and monoclonal anti-IFN-β antibodies inhibit the increase in class I HLA gene expression (HLA-B7 mRNA) in TNF-treated FS-4 cells suggesting that TNF-induced IFN-β_2 mediates the enhancing effect of TNF on HLA gene expression in human fibroblasts. The structure of this autocrine human interferon has been determined. A cDNA library was prepared from polyadenylylated RNA extracted from TNF-induced FS-4 cells, and eight IFN-β_2 cDNA clones were isolated using a 21-nucleotide synthetic oligonucleotide probe. The 1128-nucleotide sequence of IFN-β_2 mRNA and the 212-amino acid sequence of the IFN-β_2 protein were deduced from these cDNA clones. The amino acid sequences of the serologically related human IFN-β_1 and -β_2 were compared using the Sellers TT metric algorithm for locating similarities and using the pattern scoring method for evaluating the observed similarities. IFN-β_1 and -β_2 each contain a segment that is approximately 100 amino acids including 39 amino acids that are aligned and identical in the two proteins. The hydropathic index plots across these segments in the two proteins are also strikingly similar. The region of similarity between IFN-β_1 and -β_2 includes a section that is also highly conserved in all IFN-α species sequenced. Thus IFN-β_2 shares structural similarities with other human interferons that also preferentially increase class I HLA gene expression.**

Human interferons are classified as α, β, or γ based upon neutralization by appropriate type-specific antisera (1, 2). Multiple distinct β interferon (IFN-β) genes are induced by poly(I)·poly(C) in human fibroblasts (3–9). The IFN-β_1 gene has been cloned and expressed as biologically active recombinant IFN-β_1 (reviewed in refs. 10 and 11). The IFN-β_1 gene yields a 0.9-kilobase (kb) mRNA and is located on the short arm of chromosome 9 near a region that carries the IFN-α_1-related gene cluster (12, 13). The isolation of partial cDNA clones corresponding to the 1.3-kb IFN-β_2 mRNA has been reported (4). The IFN-β_2 gene is located on human chromosome 7 (9), and cloned IFN-β_2 genomic DNA transfected into rodent cells yields cells that can be induced by poly(I)·poly(C) to produce biologically active human IFN-β_2 (7, 8). Although IFN-β_1 and -β_2 mRNAs do not cross-hybridize with the respective heterologous IFN-β cDNA probes, polyclonal and monoclonal antisera raised against IFN-β_1 neutralize the anti-viral activity of IFN-β_2 (3–9). IFN-β-related DNA has also been localized on human chromosomes 2 and 4 (14, 15). Although the structural details of these loci remain to be investigated, biological data strongly suggest the presence of a functional IFN-β gene on human chromosome 2 (5, 16).

Human tumor necrosis factor (TNF) induces IFN-β_2 mRNA in human diploid fibroblasts (FS-4 strain) without the induction of any detectable IFN-β_1 mRNA (17). IFN-β_2 was also found to be involved in a complex network of interactions triggered in human fibroblast cultures by growth modulatory cytokines such as platelet-derived growth factor, interleukin-1, and IFN-β_1 (38). Endogenously produced IFN-β_2 appears to mediate the anti-viral effect of TNF in FS-4 cells since this effect can be blocked by anti-IFN-β serum (17). Endogenously produced IFN-β_2 appears also to modulate (suppress) the mitogenic effect of TNF in FS-4 cell cultures. Anti-IFN-β serum amplifies this effect suggesting that the endogenous production of IFN-β_2 represents a negative feedback mechanism in the regulation of cell proliferation (17).

Human α and β interferons preferentially increase the expression of class I histocompatibility antigens (HLA-A, -B), but not that of class II antigens (HLA-DR) in human cells (reviewed in refs. 7 and 18). In contrast, human IFN-γ preferentially increases the expression of class II HLA antigens. Like IFN-β_1 and α interferons, recombinant IFN-β_2 increases the steady-state levels of HLA-A, -B (class I) mRNAs in fibroblasts (7). This increase does not require the synthesis of another protein intermediate as it can be observed in the presence of cycloheximide (7).

Collins *et al.* (19) reported that TNF increases mRNA and protein levels of HLA-A, -B (class I) antigens but not HLA-DR (class II) antigens in cultured human endothelial cells and fibroblasts. The increase of HLA-A, -B antigens is maximal by 4 days after the beginning of TNF treatment and persists at elevated levels for at least 7 days in the continued presence of TNF. This phenomenon is of particular interest because it could represent one of the precipitating factors in microvascular thrombosis leading eventually to hemorrhagic tumor necrosis. The induction of class I HLA genes by TNF was mediated by the synthesis of another cellular protein because no HLA mRNA induction was seen in the presence of cycloheximide (19). These authors noted that the induction of class I but not class II HLA genes by TNF was similar to the effect of IFN-α and -β on these cells. These investigators failed to detect IFN mRNA accumulation in this system using the IFN-β_1 cDNA probe available to them (19).

The observation that TNF induced IFN-β_2, but not IFN-β_1, in human fibroblasts (17) suggested that IFN-β_2 was likely to be the putative "protein intermediate" postulated by Collins *et al.* (19). The TNF-induced endogenous IFN-β_2 could be the basis for the increase in HLA-A, -B gene expression. In this article we report that the increase in HLA-B7 mRNA level in TNF-treated fibroblasts can be inhibited by polyclonal and monoclonal anti-IFN-β antibod-

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Abbreviations: IFN, interferon; kb, kilobase; TNF, tumor necrosis factor.

ies suggesting that secretion of IFN-β is involved in the increase in TNF-induced HLA gene expression. Furthermore, the only IFN-β mRNA detectable in TNF-treated cells in these experiments is the 1.3-kb IFN-β_2 mRNA suggesting that it is this cytokine that mediates the increased HLA gene expression. We have determined the structure of IFN-β_2 mRNA induced by TNF in FS-4 cells and observe that the deduced amino acid sequence of IFN-β_2 shares structural similarities with characterized α and β interferons. The present functional and structural studies delineate new aspects of the intricate cytokine network that underlies key biological effects of TNF.

MATERIALS AND METHODS

Materials. Human foreskin diploid fibroblasts (FS-4 strain) were obtained from Jan Vilček (New York University School of Medicine, New York) and the human trisomy 21 fibroblasts GM2571 were obtained from The Human Genetic Mutant Cell Repository (Camden, NJ). Recombinant *Escherichia coli*-derived human TNF (specific activity, 4.8×10^7 units/mg) was a gift from The Suntory Institute for Biomedical Research (Osaka, Japan); partially purified human diploid fibroblast IFN-β (lot 4, specific activity, 2×10^6 units/mg) was obtained from Dr. Rentschler, Arzneimittel G.m.b.H, Laupheim, F.R.G.; rabbit polyclonal antiserum to partially purified human IFN-β was obtained from Edward A. Havell (Trudeau Institute, Lake Saranac, NY); murine monoclonal neutralizing antibodies to human IFN-α and -β were purchased from Boehringer Mannheim, and murine monoclonal neutralizing antibody to human IFN-γ was purchased from Interferon Sciences (New Brunswick, NJ). Cycloheximide was purchased from Sigma, and [α-^{32}P]dCTP and [γ-^{32}P]ATP were purchased from New England Nuclear. Restriction endonucleases and other enzymes used in the DNA studies were purchased from New England Biolabs.

Blot–hybridization analyses were carried out using synthetic oligonucleotide probes 5′ end-labeled with ^{32}P for IFN-β_2 mRNA (5′ GCT GGA CTG CAG GAA CTC CTT 3′, ref. 17) and HLA-B7 heavy chain mRNA (5′ GAA GCT TCT ATC TCC TGC TGG TCT G 3′, ref. 20) that are complementary to the respective mRNAs, whereas full-length, nick-translated, ^{32}P-labeled cDNA clones in pBR322 were used as probes for IFN-α_1 (pLM001, ref. 21), IFN-β_1 (pD19, ref. 22), and IFN-β_3 (pHE330, ref. 23).

cDNA Cloning and Nucleotide Sequencing. Procedures for the growth of FS-4 and GM2571 cells, for RNA extraction, blot–hybridization analyses, and autoradiography have been described (17). FS-4 cells grown in 150-mm plastic Petri dishes (Flow Laboratories) were treated with IFN-β (250 units/ml) for 15 hr followed by TNF (30 ng/ml) and cycloheximide (50 μg/ml) for 8 hr. This protocol maximizes the expression of IFN-β_2 mRNA in FS-4 cells (17, 38). Approximately 1 μg of polyadenylylated RNA extracted from these TNF-induced cells was used to prepare a cDNA library in pBR322 according to the method of Gubler and Hoffman (24). Approximately 8000 independent cDNA colonies were screened (22, 25) using the synthetic IFN-β_2 probe 5′ end-labeled with ^{32}P (17). The nucleotide sequences of the cDNA inserts in IFN-β_2-specific cDNA clones have been determined by subcloning cDNA restriction fragments into M13 vectors (26) and sequencing the single-stranded M13 DNA using the dideoxy chain-termination method (27).

Implementation of the Computer Methods for Amino Acid Sequence Comparisons. The Sellers TT algorithm (28) was implemented on a Sperry 7000 computer using a Versatec Printer to plot the output of the metric algorithm. All amino acid matches between two sequences were scored as equally important ("distance" of 0), and all mismatches were also scored as equally important ("distance" of 1). Evaluation of secondary structure conservation in an alignment and the charge or solubility properties of amino acid residues are not part of the TT analysis; thus, conservation of these features in an alignment represents an independent test of the similarity between two proteins. A specific strategy was used for evaluating the output of mathematical or metric algorithms that renders the choice of particular numerical values for the various parameters, particularly the gap or null penalty, of only transient importance (23). In essence, this strategy is based on the premise that a run of length m identities is more important than m runs of length 1 identity each. The pattern or entropy score (Π) for nucleic acid sequence alignments (23) was modified to make it applicable to amino acid sequence analyses. The description "best possible alignment" is defined in terms of the dissimilarity equation in the TT algorithm and the pattern (Π) score. The pattern score is, in principle, the equivalent of "entropy" in information theory as enunciated by Shannon (see ref. 29 for a review). The possibility that the similarity of two protein sequences is due to amino acid composition alone was evaluated by a permutation method (PERMSTAT, ref. 30), and standard programs were used to calculate hydropathic indices across proteins using several different tabulations of the relative hydrophobicities of individual amino acids (31).

RESULTS AND DISCUSSION

TNF Increases HLA-B7 and IFN-β_2 mRNA Levels in Human Fibroblasts. Polyadenylylated RNA was extracted from fibroblast cultures treated with TNF (30 ng/ml) for 4 days and analyzed for the content of the 1.5-kb heavy chain HLA-B7 mRNA by RNA gel blotting using the B7-specific synthetic oligonucleotide 5′ end-labeled with ^{32}P as the probe. Fig. 1 shows that there is a low constitutive level of HLA-B7 mRNA in confluent human fibroblasts that increased in cells treated with TNF for 4 days. This blot was then hybridized with the IFN-β_2 probe. TNF increased the level of the 1.3-kb IFN-β_2 mRNA confirming observations reported (17). In other experiments, no hybridization was detected when appropriate RNA gel blots of mRNA extracted from TNF-induced FS-4 cells were hybridized using IFN-β_1 (22), IFN-β_3 (23), or IFN-α_1 (21) probes (data not shown).

Anti-IFN-β Serum Inhibits the TNF-Induced Increase in HLA-B7 mRNA Level. The possibility that the endogenous production of IFN-β_2 in response to TNF mediates the increase in HLA-B7 gene expression was tested in an experiment in which anti-IFN-β serum was added to the cultures together with TNF. Fig. 2 illustrates an experiment in which the presence of anti-IFN-β serum in the culture medium for 4 days together with TNF inhibited the increase in HLA-B7 mRNA level. A two-dimensional laser densitometric scan of the autoradiogram in Fig. 2 indicated that anti-IFN-β serum inhibited the increase in HLA-B7 mRNA level by approximately 70%. The TNF-induced expression of the 1.3-kb IFN-β_2 mRNA is unaffected by the presence of the antiserum (Fig. 2). The inhibition by polyclonal anti-IFN-β

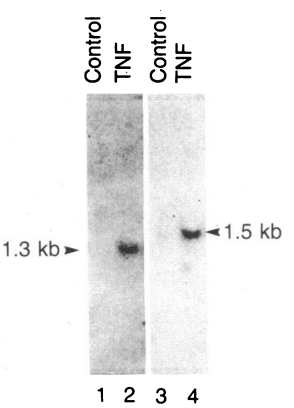

FIG. 1. Enhancement of IFN-β_2 and HLA-B7 heavy chain mRNA levels in TNF-treated human fibroblasts. Confluent cultures of human fibroblasts (GM2571, four cultures in T-75 tissue culture flasks per group), 3 days after the last change of medium, were treated with TNF (30 ng/ml) as described (17) for 4 days; and the polyadenylylated cellular RNA was extracted and analyzed by RNA gel blot hybridization using, first, the HLA-B7 heavy chain probe (lanes 3 and 4), and then the IFN-β_2 probe (lanes 1 and 2).

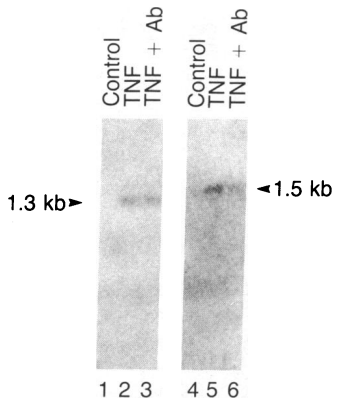

FIG. 2. Anti-IFN-β serum inhibits the TNF-induced increase in HLA-B7 gene expression. Confluent cultures of human fibroblasts (FS-4, five cultures in T-75 tissue culture flasks per group) were treated for 4 days with TNF (30 ng/ml) as described (17) in the presence or absence of anti-IFN-β serum (100 μl per flask). Polyadenylylated cellular RNA was extracted and analyzed by blot hybridization using first the HLA-B7 heavy chain probe (lanes 4, 5, and 6), and then the IFN-β_2 probe (lanes 1, 2, and 3).

serum of the TNF-induced increase in HLA-B7 mRNA level was observed in three separate experiments. Furthermore, murine monoclonal antibodies that neutralize human IFN-β, but not those that neutralize human IFN-α or -γ, also inhibit the increase in HLA-B7 mRNA content in TNF-treated fibroblasts (data not shown). Thus, the increase in HLA-B7 gene expression in TNF-treated fibroblasts appears to be, at least in part, secondary to the secretion of IFN-β. The available data (ref. 17 and the present studies) strongly suggest that the interferon species involved is IFN-β_2.

Structural Studies of TNF-Induced IFN-β_2. The structure of the endogenous cytokine IFN-β_2 has been determined, and its similarity with IFN-β_1 and IFN-α has been evaluated. A cDNA library (8000 clones) prepared from polyadenylylated RNA extracted from TNF-induced FS-4 cells was screened using the IFN-β_2 21-mer synthetic oligonucleotide probe (8, 17). Eight IFN-β_2-specific cDNA clones were isolated and characterized. Five of these had cDNA inserts $\geq$1 kb and represent cDNA clones containing the entire coding region of IFN-β_2, the entire 3'-noncoding region, and most of the 5'-noncoding region. The longest of these clones (p35) appears to contain the entire 5'-noncoding region. The strategy used for determining the nucleotide sequence of these cDNA clones is illustrated in Fig. 3. The nucleotide sequence of IFN-β_2 mRNA inferred from these studies is shown in Fig. 4. The amino acid sequence deduced from the nucleotide sequence is also shown in Fig. 4.

The 1.3-kb IFN-β_2 mRNA contains 1128 nucleotides (Fig. 4) together with a 3' poly(A) tail of 150–165 nucleotides (32). It consists of a 636-nucleotide coding region, a 63-nucleotide 5'-noncoding region and a 429-nucleotide 3'-noncoding region that contains two AAATAA polyadenylylation signals. The inference that the IFN-β_2 protein is 212 amino acid residues is consistent with the observation that the primary translation product of IFN-β_2 mRNA has an apparent molecular weight of 25 kDa (7). There is an amino-terminal 30- to 33-residue hydrophobic stretch that could act as the secretory signal sequence. Although the precise amino terminus of the mature secreted IFN-β_2 protein has not yet been determined, the inference that the mature protein is approximately 179 residues long is consistent with the observation that the processed IFN-β_2 polypeptide has an apparent molecular size of 21–22 kDa (7). The IFN-β_2 amino acid sequence reveals two potential N-glycosylation sites consistent with the observation that the IFN-β_2 secreted by human fibroblasts is glycosylated (7).

The structure of IFN-β_2 described in Fig. 4 is based on the characterization of a full-length cDNA clone isolated from a cDNA library prepared from TNF-induced fibroblasts. Zilberstein *et al.* (33) have deduced the structure of IFN-β_2 based on the characterization of partial cDNA clones isolated from a cDNA library prepared from poly(I)·poly(C)-induced fibroblasts. These investigators also located the major RNA initiation site (the 5'-cap site) in this gene by appropriate S1-nuclease protection experiments using mRNA from poly(I)·poly(C)-induced fibroblasts and ^{32}P-labeled DNA fragments derived from IFN-β_2 genomic DNA clones (33). The description of the structure of human IFN-β_2 in Fig. 4 is identical to that deduced by Zilberstein *et al.* (33). Furthermore, the location of the major RNA initiation site (the cap site) as judged by S1-nuclease protection assays (33) corresponds to the first 5' nucleotide in the cDNA clone described in Fig. 4.

Similarity Between the Amino Acid Sequences of IFN-β_1 and -β_2. The amino acid sequence corresponding to the open reading frame (187 residues long) in IFN-β_1 was compared with that corresponding to the open reading frame (212 residues long) in the serologically related IFN-β_2 using the Sellers TT algorithm and the pattern or entropy scoring method. The "best" pattern common to the two amino acid sequences is illustrated in Fig. 5. It aligns a 105-residue protein segment at the amino-terminal end of IFN-β_1 with a 92-residue segment at the carboxyl-terminal end of IFN-β_2. The pattern contains 39 residues that are aligned and identical in the two sequences. The 42-residue segment of the alignment illustrated in the upper section of Fig. 5 is remarkable because it spans a region that is highly conserved between IFN-β_1 and all the IFN-α species sequenced (11). This region contains numerous amino acid residues that are aligned and identical in all 18 or more IFN-α species sequenced as well as residues that are present in a "consensus" human IFN-α sequence (11).

The segment of the alignment illustrated in the upper section of Fig. 5 has a "distance" score of 3.85 SD better than the mean of the distribution of scores obtained when 400 permuted pairs of sequences having the same amino acid compositions and lengths as the IFN-β protein segments are compared using the standard penalty and mutation matrix in the PERMSTAT program (30). This represents an underestimate of the statistical significance of the observed alignment because it does not take into account the observation that 10 of the 16 residues aligned and identical in this 42-residue alignment between IFN-β_1 and -β_2 are also aligned and identical in all ("constant" residues) or most ("consensus" residues) of the 18 or more IFN-α sequences determined (11).

The possible similarity in the higher-order structure across the particular metric alignment between IFN-β_1 and -β_2 proteins illustrated in Fig. 5 was evaluated by plotting the Kyte–Doolittle hydropathic index across these protein seg-

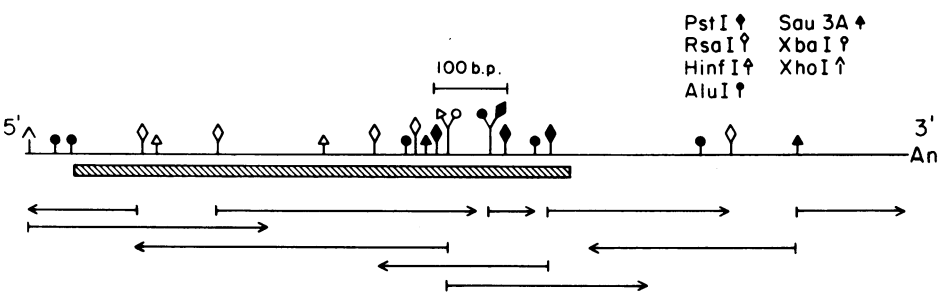

FIG. 3. Restriction map and strategy for nucleotide sequencing of the IFN-β_2 cDNA clones. The hatched bar represents the coding region, and the arrows indicate the direction of sequencing of the DNA segments.

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                                                                1
                                                                ATTCTGCCCTCGAGCCCACCGGGAACGAAAGAGAAG
CTCTATCTCCCCTCCAGGAGCCCAGCT ATG AAC TCC TTC TCC ACA AGC GCC TTC GGT CCA GTT
                            M   N   S   F   S   T   S   A   F   G   P   V
                            1   -   -   -   -   -   -   -   -  10   -   -

GCC TTC TCC CTG GGG CTG CTC CTG GTG TTG CCT GCT GCC TTC CCT GCC CCA GTA CCC
 A   F   S   L   G   L   L   L   V   L   P   A   A   F   P   A   P   V   P
 -   -   -   -   -   -   -  20   -   -   -   -   -   -   -   -   -  30   -

CCA GGA GAA GAT TCC AAA GAT GTA GCC GCC CCA CAC AGA CAG CCA CTC ACC TCT TCA
 P   G   E   D   S   K   D   V   A   A   P   H   R   Q   P   L   T   S   S
 -   -                       40                                          50

GAA CGA ATT GAC AAA CAA ATT CGG TAC ATC CTC GAC GGC ATC TCA GCC CTG AGA AAG
 E   R   I   D   K   Q   I   R   Y   I   L   D   G   I   S   A   L   R   K
                                    60

GAG ACA TGT AAC AAG AGT AAC ATG TGT GAA AGC AGC AAA GAG GCA CTG GCA GAA AAC
 E   T   C   N   K   S   N   M   C   E   S   S   K   E   A   L   A   E   N
70           *                           80

AAC CTG AAC CTT CCA AAG ATG GCT GAA AAA GAT GGA TGC TTC CAA TCT GGA TTC AAT
 N   L   N   L   P   K   M   A   E   K   D   G   C   F   Q   S   G   F   N
    90                                      100

GAG GAG ACT TGC CTG GTG AAA ATC ATC ACT GGT CTT TTG GAG TTT GAG GTA TAC CTA
 E   E   T   C   L   V   K   I   I   T   G   L   L   E   F   E   V   Y   L
        110                                     120

GAG TAC CTC CAG AAC AGA TTT GAG AGT AGT GAG GAA CAA GCC AGA GCT GTC CAG ATG
 E   Y   L   Q   N   R   F   E   S   S   E   E   Q   A   R   A   V   Q   M
            130                                     140

AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG AAA AAG GCA AAG AAT CTA GAT GCA ATA
 S   T   K   V   L   I   Q   F   L   Q   K   K   A   K   N   L   D   A   I
                150                                     160

ACC ACC CCT GAC CCA ACC ACA AAT GCC AGC CTG CTG ACG AAG CTG CAG GCA CAG AAC
 T   T   P   D   P   T   T   N   A   S   L   L   T   K   L   Q   A   Q   N
                    170     *                               180

CAG TGG CTG CAG GAC ATG ACA ACT CAT CTC ATT CTG CGC AGC TTT AAG GAG TTC CTG
 Q   W   L   Q   D   M   T   T   H   L   I   L   R   S   F   K   E   F   L
                        190                                     200

CAG TCC AGC CTG AGG GCT CTT CGG CAA ATG TAG CATGGGCACCTCAGATTGTTGTTGTTAATGG
 Q   S   S   L   R   A   L   R   Q   M
                        210     212

GCATTCCTTCTTCTGGTCAGAAACCTGTCCACTGGGCACAGAACTTATGTTGTTCTCTATGGAGAACTAAAAGTA
TGAGCGTTAGGACACTATTTTAATTATTTTTAATTTATTAATATTTAAATATGTGAAGCTGAGTTAATTTATGTA
AGTCATATTTTATATTTTTAAGAAGTACCACTTGAAACATTTTATGTATTAGTTTTGAAATAATAATGGAAAGTG
GCTATGCAGTTTGAATATCCTTTGTTTCAGAGCCAGATCATTTCTTGGAAAGTGTAGGCTTACCTCAAATAAATG
GCTAACTTTATACATATTTTTAAAGAAATATTTATATTGTATTTATATAATGTATAAATGGTTTTTATACCAATA
AATGGCATTTTAAAAAATTC-poly(A)
                    1128
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FIG. 4. Complete nucleotide sequence of IFN-β_2 cDNA and its deduced amino acid sequence. The amino-terminal hydrophobic stretch that may act as the secretory signal sequence is indicated with a dashed line. The two potential N-glycosylation sites are indicated by an asterisk next to the asparagine (N) residue.

ments (31, 34). Fig. 6 shows one of several hydrophobicity analyses carried out. A subset of amino acids that are aligned and identical between the two sequences in Fig. 5 are joined by dotted lines in Fig. 6. There is a striking similarity between the hydrophobicity profiles across the two protein segments.

The particular alignment described in Fig. 5 between the amino-terminal portion of IFN-β_1 and the carboxyl-terminal portion of IFN-β_2 is consistent with data concerning the biologically active regions of these molecules. It has been suggested, based on several different kinds of experiments, that the amino-terminal 60–80 residues of IFN-α_2 (and by inference, of IFN-β_1) interact with the high-affinity cell surface receptor for IFN-α/β (35–37). Thus, the region of greatest similarity between IFN-β_1 and -β_2, depicted in Fig. 5, aligns a portion of IFN-β_2 with a putative receptor-binding region in IFN-β_1.

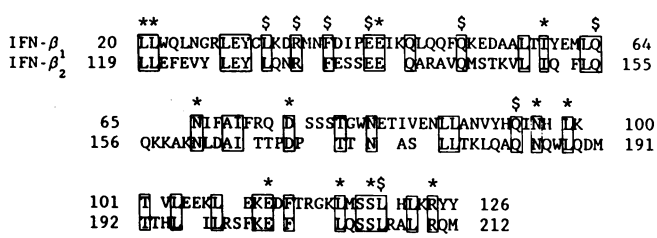

FIG. 5. Similarity between IFN-β_1 and -β_2 amino acid sequences. The "best" common pattern between the amino acid sequences of IFN-β_1 and -β_2 was determined using the Setters TT algorithm and the pattern score. The symbol \$ identifies residues that are aligned and identical in IFN-β_1 and all IFN-α species sequenced to date (11), whereas the symbol * identifies residues in IFN-β_1 that are also part of an IFN-α consensus sequence (11).

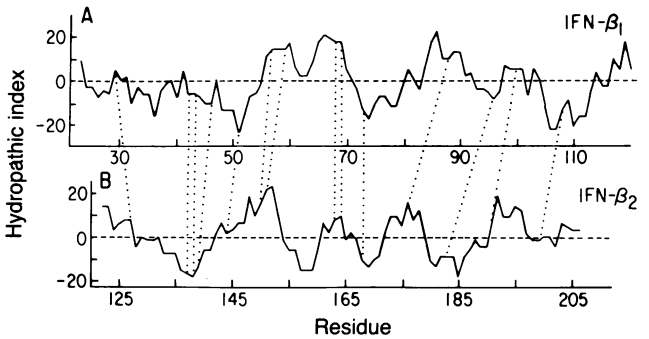

FIG. 6. Plot of the hydropathic index across the alignment between IFN-β_1 and -β_2 in Fig. 5. In this illustration the Kyte–Doolittle hydrophobicity values (34) were used averaged over seven residues. Dotted lines compare numerous residues that are aligned and identical in Fig. 5.

The high-affinity cell-surface receptor for α and β interferons is coded for by a gene on human chromosome 21 (reviewed in ref. 7). Recombinant IFN-β_2 is biologically active in human–rodent hybrids in which chromosome 21 is the only human chromosome present (7) suggesting that IFN-β_2 may interact with the same cell-surface receptor used by the other type I interferons. The structural similarity detected by us between IFN-β_2 and IFN-β_1 supports this possibility. This similarity also suggests a basis for the serologic cross-neutralization between the two proteins.

The action of TNF-induced IFN-β_2 described in this article, namely the increased expression of class I HLA antigens, is likely to contribute to the *in vivo* effects of TNF. The increase in cell-surface HLA in specific tissues may enhance the participation of the immune system, especially of cytotoxic immune cells, in the destruction of neoplastic tissue. It will be important to evaluate the role of endogenous IFN-β_2 in the pathophysiology of tumor necrosis, inflammation, and autoimmune disease.

Conclusions. The cDNA for human IFN-β_2, a cytokine whose expression is increased by tumor necrosis factor, platelet-derived growth factor, IFN-β_1, and interleukin-1 (17, 33, 38), has been cloned and characterized. The primary amino acid sequence of IFN-β_2, deduced from its cDNA clone, shares distinct similarities with that of IFN-β_1 across a region in IFN-β_1 that is also highly conserved among all the IFN-α species characterized. IFN-α, -β_1, and -β_2 are known to preferentially increase the expression of class I histocompatibility antigens (HLA-A, B) in human fibroblasts. Experiments described in this article suggest that the ability of TNF to induce endogenous IFN-β_2 in human fibroblasts accounts for the increase in HLA gene expression observed in TNF-treated cells. IFN-β_2 has emerged as an autocrine interferon that can mediate as well as modulate the diverse biological effects of several other cytokines.

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