Interferon-κ, a Novel Type I Interferon Expressed in Human Keratinocytes*

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High throughput cDNA sequencing has led to the identification of interferon-κ, a novel subclass of type I interferon that displays ~30% homology to other family members. Interferon-κ consists of 297 amino acids, including a 27-amino acid signal peptide and a series of cysteines conserved in type I interferons. The gene encoding interferon-κ is located on the short arm of chromosome 9 adjacent to the type I interferon gene cluster and is selectively expressed in epidermal keratinocytes. Expression of interferon-κ is significantly enhanced in keratinocytes upon viral infection, upon exposure to double-stranded RNA, or upon treatment with either interferon-γ or interferon-β. Administration of interferon-κ recombinant protein imparts cellular protection against viral infection in a species-specific manner. Interferon-κ activates the interferon-stimulated response element signaling pathway and a panel of genes similar to those regulated by other type I interferons including anti-viral mediators and transcriptional regulators. An antibody that neutralizes the type I interferon receptor completely blocks interferon-κ signaling, demonstrating that interferon-κ utilizes the same receptor as other type I interferons. Interferon-κ therefore defines a novel subclass of type I interferon that is expressed in keratinocytes and expands the repertoire of known proteins mediating host defense.

Interferons (IFNs)1 are a family of functionally related cytokines that confer a range of cellular responses including antiviral, antiproliferative, antitumor, and immunomodulatory activities (1, 2). They are classified as type I or type II according to their structural and functional properties. Although the sole member of the type II family is IFN-γ, there are multiple members of the type I interferon class, which is divided into the IFN-α, IFN-β, and IFN-ω subclasses (1, 2). In humans, excluding pseudogenes, there are 13 non-allelic IFN-α genes, a single β gene, and a single ω gene. Members of the IFN-α family display greater than 80% identity to each other, IFN-ω displays ~60% identity to IFN-α, and IFN-β is ~40% identical to the other family members. The evolutionary conservation of the type I IFN genes is reflected in their common intron-less structure and their co-localization to the short arm of chromosome 9, which suggest that type I IFNs arose by gene duplication (3). The subtypes were initially categorized further by their cell of origin. IFN-α and IFN-ω genes were thought to be produced predominantly by leukocytes and IFN-β by fibroblasts. However, upon appropriate induction, most human cell types can generate type I IFNs (2). Exposure to a variety of agents triggers the rapid and transient production of type I IFNs, with viruses being the most efficient natural inducers (4, 5). Certain bacteria can also induce expression, as can double-stranded RNA (dsRNA) and endotoxin. In contrast, trophoblast IFNs or IFN-τ, which are found only in ruminant ungulate species, are not induced by viral challenge (6). These genes are expressed by the embryonic trophoectoderm at a specific time during early pregnancy, and though they have the typical properties of other type I IFNs, their major function is to create conditions for the completion of pregnancy (6).

Despite the diversity in their sequence, all type I IFNs employ a common type I IFN receptor complex (IFNAR) that is composed of two chains, a 135-kDa subunit (IFNAR1) and a 115-kDa subunit (IFNAR2c) (7–9). IFN-induced receptor dimerization of the IFNAR1 and IFNAR2c chains initiates a signaling cascade that involves tyrosine phosphorylation of the Tyk2 and Jak1 tyrosine kinases and subsequent phosphorylation of the STAT1 and STAT2 proteins (10, 11). Association of the phosphorylated STAT proteins with the p48 DNA binding subunit forms the interferon stimulated gene factor 3 multimeric complex, which translocates to the nucleus and binds to interferon-stimulated response elements (ISRE) found upstream of interferon-inducible genes. IFN signaling culminates in the modulation of a wide range of cellular responses including anti-viral activity, tumor anti-proliferation, enhancement of natural killer cell activity, and induction of major histocompatibility complex antigen expression (1, 2, 10, 11). The cellular activities of IFNs have attracted much interest for clinical applications, with IFNs now being used to treat a broad range of diseases including multiple sclerosis, leukemia, and hepatitis (2, 12). We report here the identification and characterization of a novel subclass of the type I IFN family that we have named interferon-κ, which is expressed in keratinocytes, signals through the type I receptor complex, and mediates anti-viral activity.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) AF315688, AF384048, and AF384047.

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1 The abbreviations used are: IFN, interferon; dsRNA, double-stranded RNA; IFNAR, type I IFN receptor complex; STAT, signal transducer and activator of transcription; ISRE, interferon-stimulated response element; EST, expressed sequence tag; PCR, polymerase chain reaction; bp, base pairs; EMCV, encephalomyocarditis virus; ELISA, enzyme-linked immunosorbent assay; OAS, 2–5A oligoadenylatesynthetase; PKR, dsRNA-dependent protein kinase; kb, kilobase pair; HEK, human embryonic kidney.

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Experimental Procedures

Isolation of IFN-κ DNA and Gene Sequence—The Human Genome Sciences expressed sequence tag (EST) data base of ~3 million cDNA sequences derived from over 900 human cDNA libraries was screened for homologies of the interferon family using the BLAST algorithm. A single EST (HKAP151) with significant type I interferon homology was identified and sequenced completely to reveal an open reading frame of 207 amino acids. The amino acid sequence has been deposited in GenBankTM under accession number AF315686. The IFN-κ genomic region was generated in two steps. The genomic sequence that contains the IFN-κ coding region was PCR-amplified from human genomic DNA using oligonucleotide primers (GTGCCAGAGATTTTGCTGGA and GTACATTCCAGATAATTTCCA) that correspond to nucleotides 472–494 and 2232–2252 in Fig. 1. The upstream promoter region was isolated by PCR amplification from genomic DNA digested with restriction sites. The resulting amplicon was restriction digested with BglII and Asp718 and subcloned into like-digested pHE4, a bacterial expression vector derived from pC4. The resulting plasmid was transformed into chemically competent E. coli strain [DH5α] and the cDNA inserted between the SalI and NorI sites of pCMV-Sport2 (Invitrogen) and was used as template for the SP6-driven transcription coupled translation reticulocyte lysate system (Promega) according to the manufacturer’s instructions.

Identification of the NH2-terminal Cleavage Site—Chinese hamster ovary cells were stably transfected with pc4/IFN-κ using LipofectAMINE (Invitrogen). Stable cell lines expressing IFN-κ mRNA as determined by Taqman analysis were expanded, and 100 ml of conditioned supernatant was collected. Secreted IFN-κ was captured by Poros HS-50 cation exchange chromatography at pH 6.0. IFN-κ was eluted using a salt gradient of 0 to 1.5 M sodium chloride. By SDS-polyacrylamide gel electrophoresis, a protein of molecular mass of ~30 kDa was observed for samples that eluted between 0.6 and 0.8 M NaCl. Several fractions were taken and rechromatographed on a polyacrylamide gel, and the NH2 terminus sequence was determined using an ABI-494 sequencer (Applied Biosystems).

Chromosomal Mapping—A panel of 24 monochromosomal somatic cell hybrids was obtained from Quantum Biotechnologies, and the G3 panels of 53 radiation hybrids were obtained from Research Genetics. The following oligonucleotides, which span a 600-bp region of the IFN-κ coding region, were used for polymerase chain reaction analysis, CGTCGGAGATTTTGCTGGA and CTCTGGATTCTTCCACTCGGACA (3′ primer). 35 cycles of polymerase chain reaction (94 °C for 30 s, 58 °C for 45 s, and 72 °C for 1 min) were performed on 100 ng of each hybrid in a 50-μl reaction. Analysis of the radiation hybrid data was performed using the Stanford Human Genome Center radiation hybrid server.

Keratinocyte Cultures—Primary keratinocytes (Clonetics, San Diego, CA) were cultured in serum-free medium (KGM-2 medium; Clonetics). Second- or third-passage keratinocytes at 70% confluence were cultured in serum-free medium (KGM-2 medium; Clonetics). The following oligonucleotides, which span a 600-bp region of the IFN-κ coding region, were used for polymerase chain reaction analysis, CGTCGGAGATTTTGCTGGA and GTACATTCCAGATAATTTCCA) that correspond to nucleotides 472–494 and 2232–2252 in Fig. 1. The upstream promoter region was isolated by PCR amplification from genomic DNA digested with restriction sites. The resulting amplicon was restriction digested with BglII and Asp718 and subcloned into like-digested pHE4, a bacterial expression vector derived from pC4. The resulting plasmid was transformed into chemically competent E. coli strain [DH5α] and the cDNA inserted between the SalI and NorI sites of pCMV-Sport2 (Invitrogen) and was used as template for the SP6-driven transcription coupled translation reticulocyte lysate system (Promega) according to the manufacturer’s instructions.

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Anti-viral Assay—Normal human dermal fibroblasts were seeded to an initial density of 2 × 104 cells/well in Dulbecco's modified Eagle's medium/10% fetal bovine serum in flat bottom 96-well plates and were allowed to grow to 95% confluence. Serial dilutions of recombinant IFN-κ were added to the wells. Following 24 h of incubation, EMVC 2 × 104 TCID50 was added to each well. Following an additional 24 h incubation, the cell monolayers were washed twice with phosphate-buffered saline and stained with 1% crystal violet in 15% ethanol. Staining of the plates was accomplished by extraction of stained cells with 70% ethanol/1% acetic acid. Absorbance determination was performed at 580 nm in a 96-well format ELISA plate reader. Data are expressed as absorbance versus protein concentration.

ISRE Assay—Five tandem copies of the ISRE element (TAGTT-CATTTTGCC), which mediates type I interferon-inducible expression of the interferon-inducible gene ISG54 (18) and a basal promoter containing a TATA box contained within the PBSr-Luc plasmid (catalog...
RESULTS

Isolation and Structure of IFN-α Gene—BLAST analysis of a data base of over 3 million human EST sequences identified a single EST derived from a keratinocyte library displaying novel homology to the type I interferons. Complete sequence analysis of this 1.1-kb cDNA clone revealed an open reading frame of 207 amino acids with significant homology to the other sub-classes of type I IFN and that we have named IFN-α. Although the first in-frame methionine (Met1) is designated as the initiating methionine, the possibility that the methionine at amino acid position 7 (Met7) is the initiating methionine cannot be ruled out. Neither the nucleotide context of Met1 (AAAAAAA-UGA) nor Met7 (CCUGAUAUGA) compare favorably with the strong consensus Kozak translational initiating sequence (GCCACCAUGG) (13). To confirm the sequence of the cDNA clone and to identify the genomic structure of IFN-α, the IFN-α gene was isolated and sequenced (Fig. 1). In addition to the gene-encoding sequences, ~0.5 kb of upstream sequence including the putative promoter sequence and 1 kb of downstream sequence were isolated. The genomic sequence confirmed the open reading frame sequence identified in the cDNA sequence and also revealed the presence of an intron within the 3′ untranslated region immediately following the stop codon (Fig. 1). The presence of an intron in type I IFN genes has not been reported previously (1, 2). Inspection of the promoter region revealed the presence of a putative TATA element and within 200 bp of the transcriptional start site, three GAAANN elements that have been demonstrated to mediate the viral inducibility of other interferon genes (19) are boxed. Three putative GAAANN elements that have been demonstrated to mediate the viral inducibility of other interferon genes (19) are boxed.

Comparison of the IFN-α protein sequence with the three members was performed using mature protein sequences.
Novel Type I Interferon Expressed in Keratinocytes

Existing subgroups of human type I IFNs (Fig. 2A) reveals homology throughout the coding region, including within the five α-helical regions defined in other type IFNs (20–22). Like other IFNs, IFN-κ is predicted to be secreted based on PSORT and SignalP algorithm analysis (23) with cleavage anticipated to be between amino acids Ser27 and Leu28. In vitro transcription and translation of the IFN-κ cDNA revealed a protein that migrates at ~30 kDa (Fig. 2B), somewhat larger than the anticipated molecular mass of 25.2 kDa. In the presence of microsomal membranes the IFN-κ protein is processed to remove its NH2-terminal signal peptide (Fig. 2B). To confirm that IFN-κ is secreted from mammalian cells, the full-length IFN-κ open reading frame was expressed in Chinese hamster ovary cells. Conditioned supernatant collected from IFN-κ-expressing Chinese hamster ovary cell lines was subjected to ion exchange chromatography, and a protein of the anticipated molecular mass for IFN-κ was isolated. Amino acid sequencing of the captured protein revealed identity to IFN-κ and an NH2 terminus of LDCNL, therefore confirming that IFN-κ is secreted and that cleavage occurs between Ser27 and Leu28. Although it appears that IFN-κ utilizes a signal peptide relatively longer than other type I IFNs (Fig. 2A), as discussed above, it cannot be ruled out that Met3 is the start methionine resulting in a signal peptide closer in length to that of the other type I interferon family members.

Within the 180-amino acid mature protein (Leu26–Lys207), IFN-κ demonstrates 30–52% identity to the other type I IFNs (Fig. 2C) and thus defines a novel subclass of type I IFN. Perhaps the most significant structural difference between IFN-κ and the other type I IFNs is the length of the CD loop region where IFN-κ has an insertion of 12 amino acids (Fig. 2A). This also accounts for the larger size of mature IFN-κ (180 amino acids) compared with the IFN-α (165–166 amino acids), IFN-β (166 amino acids), and IFN-ω (172 amino acids) subclasses. The mature protein contains five cysteines, and on the basis of homology and modeling to other IFNs it is expected that Cys3 forms a disulfide bond with Cys102, whereas Cys32 forms a disulfide bond with Cys167, leaving Cys162 as an unpaired cysteine. Unlike human IFN-β and IFN-ω, but in common with most type I IFN-κ species (22, 24), IFN-κ does not contain a consensus sequence for N-linked glycosylation.

To determine the chromosomal position of the IFN-κ gene, a panel of monochromosomal somatic cell hybrids retaining individual chromosomes was screened using IFN-κ-specific primers. A PCR product was detected in chromosome 9, and no amplification was observed in any other sample (Fig. 3). To sublocalize IFN-κ on chromosome 9, a panel of 83 radiation hybrids was used. We observed amplicons in hybrids 13, 15, 25, 28, 35, 40, 48, 66, and 74. Analysis of this data using the Stanford Human Genome Center radiation hybrid server revealed linkage to the SHGC-36542 marker on chromosome 9, which lies between markers D9S161 and D9S1853 on the physical map of chromosome 9. Superposition of this position with the cytogenetic map of human chromosome 9 allowed the assignment of IFN-κ to chromosomal band 9p21.2. Analysis of the recently deposited human genome sequence confirmed the map position of IFN-κ to this region on chromosome 9. It has been demonstrated previously that the IFN-α, IFN-β, and IFN-ω genes are closely linked within 400 kb in the 9p21-p22 region (3). Based on the radiation hybrid mapping and genomic sequence information, IFN-κ is located ~6.5 megabases proximal to the centromere relative to the existing type I IFN cluster.

IFN-κ is Expressed in Keratinocytes and Induced by dsRNA, Viral Infection, and by Other IFNs—Analysis of the Human Genome Sciences data base, which comprises sequences derived from ~900 independent human cDNA libraries generated from both normal and disease tissue and cell types, revealed expression of IFN-κ only in keratinocytes suggesting that IFN-κ exhibits a restricted pattern of expression. No detectable expression of IFN-κ was observed in an analysis of a panel of Northern blots containing RNA from a wide range of human cell and tissue types including brain, kidney, spleen, liver, tonsil, heart, small intestine, colon, placenta, and testis. Real-time Taqman PCR performed on a range of purified cell populations including T and B lymphocytes, monocytes, dendritic cells, endothelial cells, fibroblasts, and keratinocytes confirmed expression of IFN-κ in keratinocytes and revealed a lower level of expression in dendritic cells and monocytes but failed to detect significant expression elsewhere (data not shown).

Northern analysis performed on adult keratinocytes confirmed the expression of a 1.1-kilobase IFN-κ mRNA transcript (Fig. 4A). Expression of the IFN-κ mRNA was observed in multiple independent adult keratinocyte donor populations and also in neonatal keratinocytes (data not shown). In contrast to IFN-κ expression, IFN-β mRNA was undetectable in resting keratinocytes (Fig. 4A). Treatment of keratinocytes with dsRNA, a known inducer of IFN expression, resulted in the expected increase in IFN-β expression and also an up-regulation of IFN-κ mRNA expression (Fig. 4A).

To determine whether keratinocytes express detectable levels of IFN-κ protein, an ELISA was developed for IFN-κ. The specificity of the ELISA was demonstrated by an inability of the ELISA or the component polyclonal or monoclonal IFN-κ antibodies to cross-react with either recombinant IFN-α or IFN-β. Supernatants collected from three donor populations of adult keratinocytes cultured for 24 h revealed expression of IFN-κ at the 150–200 pg/ml level. In contrast, no IFN-κ protein was detectable in primary cell cultures of fibroblasts or in peripheral blood mononuclear cells.

The observation that dsRNA enhances IFN-κ expression suggests that IFN-κ will also be up-regulated upon viral infection. Real-time PCR analysis of keratinocytes infected with EMCV demonstrated that the IFN-κ mRNA is induced ~10-fold h after viral infection (Fig. 4B). To determine whether expression of IFN-κ is regulated by other interferons, cultured human keratinocytes were treated with IFN-γ or IFN-β. As shown in Fig. 4C, both IFN-γ and IFN-β direct a significant increase in the level of IFN-κ mRNA expression with both inducing an approximate 20-fold increase in the IFN-κ mRNA level after 3 h. The kinetics of the IFN-κ mRNA response to the two interferons, however, is somewhat different, with IFN-β mediating a more rapid response (an approximate 8-fold increase in the level of IFN-κ after 1 h) compared with the response to IFN-γ treatment (an approximate 10-fold increase after 15 h).

IFN-κ Displays Species-specific Antiviral Activity—A hallmark of all interferons is the ability to elicit anti-viral protection (1, 2). To determine whether IFN-κ has this activity, re-
FIG. 4. IFN-α mRNA expression profile. A, Northern analysis of IFN-α and IFN-β mRNA expression in untreated and dsRNA-treated keratinocytes. Equal loading of RNA was verified by 18 S rRNA analysis. B, Taqman real-time PCR analysis of IFN-α mRNA expression in EMCV-infected keratinocytes. Levels of IFN-α mRNA are expressed relative to the level observed in uninfected keratinocytes. C, Taqman real-time PCR analysis of IFN-α mRNA expression in cytokine-treated keratinocytes. Keratinocytes either untreated or treated with IFN-β or IFN-γ for 1, 5, 15, and 30 h were harvested and processed as described under “Experimental Procedures.” The level of IFN-α mRNA in each sample was determined by Taqman real-time PCR and is expressed relative to the 18 S rRNA level (× 10^-1).
IFN-α represents a novel subclass of type I interferon that is selectively expressed in keratinocytes and is ~30% identical to the other type I interferon family members. The gene encoding IFN-α is located on the short arm of chromosome 9, adjacent to the type I IFN cluster, but is relatively proximal to the centromere. In contrast to the other interferons, which are devoid of introns (1), IFN-α contains an intron within the 3’ untranslated region. In addition, the IFN-α protein is somewhat larger than the other type I IFNs because of a 12-amino acid insertion between the predicted C and D α-helices. Taken together, the gene location and structure suggest that IFN-α evolved separately from the other type I interferons. Analysis of available cDNA and genomic sequences from other species has failed to identify an ortholog of IFN-α suggesting it may have evolved later to play a specific role in humans or primates. Clearly confirmation of this will require a detailed search for orthologs of IFN-α. The relevance of the relatively long CD loop in IFN-α is unclear but perhaps influences cellular location, association with binding proteins, interaction with its cognate receptor(s), and subsequent downstream signaling. Despite its sequence and structural differences, IFN-α does employ the common IFN receptor and activates the ISRE signal transduction pathway activated by other type I IFNs. Whether IFN-α utilizes additional receptors remains to be determined.

The emergence of IFN-α expands the repertoire of human type I IFNs into four distinct subgroups, IFN-α, IFN-β, IFN-ω, and IFN-κ. An additional subclass, IFN-τ, has been identified in ruminant species (5), and more recently, limitin, a mouse gene distantly related to the type I IFNs that also signals through the common type I IFN receptor, was isolated (27). Although the type I IFNs mediate many similar biological activities, they do exhibit significant differences in the relative potency of their activities and some different immunomodulatory effects (1, 2, 10, 11, 28–32). Presumably differences in signaling downstream from the receptor (11–12, 33) combined with differences in their spatial and temporal expression dictate specificity of function for each type I IFN subgroup. Considering most type I IFNs are predominantly expressed only upon viral infection or cellular challenge, the expression of IFN-α protein in resting keratinocytes and cell types of the innate immune system (monocytes and dendritic cells) is an important characteristic of this subclass. Earlier studies also demonstrated the existence of a type I IFN (possibly IFN-κ) distinct from IFN-α or IFN-β that is expressed in uninfected keratinocytes (34). In contrast, neither IFN-α nor IFN-β are expressed in unstimulated keratinocytes, although IFN-β protein is detectable in culture supernatants of activated keratinocytes (35). In view of the critical role played by skin as primary defense organ the expression of IFN-κ in resting keratinocytes may provide a novel mechanism of host defense that will require further evaluation.

IFN-κ is capable of mediating cellular protection against at least two families of RNA viruses although it should be emphasized that the level of anti-viral activity of IFN-κ displayed against EMCV infection of fibroblasts is relatively weak compared with other type I interferons (1, 31). Further studies will be required to deter-
mine whether other viruses, including those that infect skin such as the herpes and papillomaviruses, are more susceptible to IFN-κ. Upon viral infection of keratinocytes or treatment with dsRNA, the expression of IFN-κ is further enhanced supporting a role in host defense. The observation that both IFN-β and IFN-γ also significantly increase IFN-κ expression suggests a role for IFN-κ in sustaining the host interferon response. An analysis of the IFN-κ promoter, including the three putative virus-inducible GAAANN elements (19) identified in this study, will aid in uncovering the molecular mechanisms that direct IFN-κ expression in keratinocytes and regulate its response to IFN-β, IFN-γ, and viral infection.

In addition to imparting anti-viral activity, interferons mediate a wide range of other cellular effects through the activation of a wide spectrum of interferon-inducible genes. These activities include inhibition of proliferation of normal and tumor cells, stimulation of natural killer cells, enhancement of major histocompatibility complex antigen expression, and the stimulation of tumor antigens (1, 2, 26, 36). The observation that IFN-κ utilizes the common IFN receptor and activates the ISRE, as evidenced by its ability to activate transcriptional activation of the ISG54 ISRE, suggests that it will elicit activities similar to those elicited by other interferons. Indeed, initial transcriptional profiling demonstrates that IFN-κ activates the three well defined anti-viral pathways mediated by PKR, OAS, and Mx proteins. IFN-κ also up-regulates the transcription factors STAT-1 and IRF-1, which play integral roles in mediating the interferon response (11). Mice lacking the STAT1 gene have no innate response to either viral or bacterial infection due to the disruption of the ISG response (37), whereas IRF-1 regulates the expression of many inducible genes including major histocompatibility complex class I antigens (11, 38). The observation that IFN-κ is induced by the inflammatory mediator IFN-γ, and also other type I interferons, further supports involvement of IFN-κ in host defense. Clearly, further analysis will be required to determine how the range and potency of cellular activities mediated by IFN-κ compares to the effects of other type I interferons and to determine the contribution IFN-κ makes to host defense and cellular maintenance, particularly in the skin and within the innate immune system.

The therapeutic utility of IFN-κ should also be considered. Existing type I interferons have been used successfully to treat a range of diseases such as various forms of leukemia, hepatitisis, and multiple sclerosis (2, 12). However, existing IFN therapies do elicit side effects, including fever, fatigue, anorexia, and flu-like symptoms. In addition their efficacy may be limited by the production of neutralizing antibodies (12, 30, 31). Although studies will be required to establish clinical utility it is plausible that IFN-κ could provide an alternative interferon treatment to complement existing type I interferons in the clinic, by either expanding utility and/or by reducing undesirable side effects.

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REFERENCES
1. Pestka, S., Langer, J. A., Zoon, K. C., and Samuel, C. E. (1987) Annu. Rev. Biochem. 56, 727–777
2. Maeyer, E., and Maeyer-Guignard, J. (1998) in The Cytokine Handbook (Thompson, A., ed) 3rd Ed., pp. 491–516, Academic Press, San Diego, CA
3. Diaz, M. O., Pomylka, H. M., Bolandner, S. K., Maltepe, E., Malik, K., Brownstein, R., and Olapade, O. I. (1994) Genomics 22, 540–552
4. Cavalieri, R. L., Havel, E. A., Vilecek, J., and Pestka, S. (1977) Proc. Natl. Acad. Sci. 74, 4415–4419
5. Raj, N. B. K., and Pitha, P. M. (1983) Proc. Natl. Acad. Sci 80, 3923–3927
6. Bartsch, R. M., Ealy, A. D., Alexzeno, A. P., Han, C. S., and Erzaki, T. (1999) Placenta 20, 259–264
7. Uez, G., Lutfalla, G., and Gresser, I. (1990) Cell 60, 225–234
8. Sol, J., Mariano, T. M., Lim, J. K., Izotova, L., Mirochnitchenko, O., Schwartz, B., Langer, J. A., and Pestka, S. (1994) J. Biol. Chem. 269, 18102–18110
9. Domanski, P., Witte, M., Kellum, M., Rubinstein, M., Hackett, R., Pitha, P., and Colamunici, O. R. (1995) J. Biol. Chem. 270, 21606–21611
10. Schindler, C., and Darnell, J. E. (1995) Ann. Rev. Biochem. 64, 621–651
11. Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., and Schreiber, R. D. (1998) Annu. Rev. Biochem. 67, 227–264
12. Gutterman, J. U. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1198–1205
13. Kozak, M. (1989) J. Cell. Bio 108, 229–241
14. Subramani S., Mulligan, R., and Berg, P. (1981) Mol. Cell. Biol. 1, 749–755
15. Dillon, P. J., and Rosen, C. A. (1990) J. Cell. Bio 119, 259–272
16. Gentz, R. L., and Coleman, T. A. (2001) U. S. Patent 6,194,168, February 27, 2001
17. Lin, L. S., Yamamoto, R., and Drummond, R. J. (1986) Methods Enzymol. 119, 183–192
18. Levy, D. E., Kessler, D. S., Pine, R., Reich, N., and Darnell, J. E. (1988) Genes Dev. 2, 383–388
19. Pitha, P. M., Au, W.-C., Lowther, W., Juang, Y.-T., Schafer, S. L., Bursky, L., Hiscott, J. and Moore, P. A. (1998) Biochimie (Paris) 80, 651–658
20. Senda, T., Saitoh, S., and Mizu, T. (1995) J. Mol. Biol. 25, 187–207
21. Badhurshah, S., Foster, L. L., Hura, R., Reichert, P., Trotta, P. P., Nagabushan, T. L., and Walter, M. R., (1996) Structure 4, 1453–1463
22. Karpusas, M., Noble, M., Bentley, C. B., Meier, W., Lipscomb, W. N., and Goelz, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11813–11818
23. von Heine, G. (1986) Nucleic Acids Res. 14, 4683–4690
24. Pestka, S. (1986) Methods Enzymol. 119, 3–14
25. Domanski, O. R., and Dummanski, P. (1993) J. Biol. Chem. 268, 10895–10899
26. Gao, Y., Xue Sa., and Griffin, B. E. (1999) Mol. Cell. Biol. 19, 7305–7313
27. Orito, K., Medina, K. L., Tomiyama, Y., Ishikawa, J., Okajima, Y., Ogawa, M., Yokota, T., Aoyama, K., Takahashi, I., Kincade, P. W., and Matsuzawa, Y. (2000) Nat. Med. 6, 659–666
28. Kiefer, M., Rubinstein, M., and Pestka, S. (1981) Arch. Biochem. Biophys. 210, 319–329
29. Fish, E. N., Banerjee, K., and Stebbing, N. (1983) Biochem. Biophys. Res. Commun. 129, 537–546
30. Oraltio, J. R., Herberman, R. B., Harvey, C., Osheroff, P., Pan, Y.-C., Kelder, B., and Pestka, S. (1984) Proc. Natl. Acad. Sci. 81, 4926–4929
31. Adolf, G. R. (1995) Mult. Scler. 1, 44–47
32. Pestka, S. (2000) Biopolymers 55, 254–287
33. Der, S. D., Zhou, A., Williams, R. R., and Silverman, R. H. (1998) Proc. Natl. Acad. Sci. 95, 15623–15628
34. Yaar, M., Pallaroni, A. V., and Gilchrist, B. A. (1986) J. Cell Biol. 103, 1349–1354
35. Fujisawa, H., Kondo, S., Wang, B., Shiyivi, G. M., and Sauder, D. N. (1997) J. Interferon Cytokine Res. 17, 721–725
36. Greiner, J. W., Guadagni, F., Naguchi, P., Pestka, S., Colcher, D., Fisher, P. B., and Schliom, J. (1987) Science 235, 895–898
37. Meraz, M. A., White, J. M., Sheehan, K. C., Bach, E. A., Rodig, S. J., Dighe, A. S., Kaplan, D. H., Riley, J. K., Greenlund, A. C., Campbell, D., Carver-Moore, K., DuBois, R. N., Clark, R., Aguett, M., and Schreiber, R. D. (1996) Cell 84, 431–442
38. Duncan, G. S., Mittrucker, H. W., Kagi, D., Matsuyama, T., and Mak, T. W. (1990) J. Exp. Med. 184, 2923–2948
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