A Family of Genes Coding for Two Serologically Distinct Chicken Interferons*

Christine Sick, Ursula Schultz, and Peter Staeheli†

From the Abteilung Virologie, Institut für Medizinische Mikrobiologie und Hygiene, University of Freiburg, 79008 Freiburg, Federal Republic of Germany

Southern blot analysis and screening of a genomic λ phage library with the previously cloned chicken interferon (IFN) cDNA indicated that the chicken genome contains at least 10 IFN genes. A particularly strongly hybridizing phage clone that we analyzed in more detail carried a head to tail arrangement of three intron-less IFN genes that differed from each other and from the cloned chicken IFN cDNA by only a few base changes. The primary translation products of these three IFN genes consist of 193 amino acids, and the mature proteins are composed of 162 amino acids. All three genes of this IFN family, designated IFN1, yielded active chicken IFN when expressed individually in transfected COS7 cells. A weakly hybridizing phage clone contained an additional intron-less chicken IFN gene, designated IFN2, whose product was 57% identical to chicken IFN1. Southern blot analysis suggested that the chicken genome contains a single IFN2 gene. The primary translation product of IFN2 consists of 203 amino acids, and the mature protein is composed of 176 amino acids. Purified recombinant chicken IFN2 from Escherichia coli had a specific antiviral activity of about 10^6 units/mg, which was about 20-fold lower than that of chicken IFN1 purified in parallel. The antiviral activity of chicken IFN2 from E. coli or from transfected COS7 cells could not be neutralized by antiserum to recombinant chicken IFN1. Thus, like mammals, the chicken has a large number of type I IFN genes that code for at least two serologically distinct antiviral activities.

In mammals, cytokines with antiviral activity are classified as type I and type II interferons (IFNs). Type I IFN includes α-, β-, ω-, and τ-IFNs, which have related structures and use a common receptor. The former three are synthesized in response to virus infection (1, 2), whereas τ-IFN is synthesized in response to developmental stimuli in the trophoblast of ruminants (3) and humans (4). Mature α- and τ-IFNs of humans consist of 172 amino acids, whereas α- and β-IFNs are composed of 165 or 166 amino acids (2). Antisera prepared against α-IFN or β-IFN showed a high degree of subtype specificity and did not neutralize the antiviral activity of ω-IFN (5). All mammalian type I IFNs are coded for by intron-less genes (2). The α- and ω-IFNs are encoded by gene families with as many as 20 closely related members (2). In the various species, the β-IFNs are coded for by single genes (e.g. mouse) or by gene families (e.g. cattle). Type II IFN or IFN-γ is synthesized by antigen- or mitogen-stimulated T cells (1). It is encoded by a single gene with introns, has pleiotropic regulatory effects on cells of the immune system (6, 7), and is the principal macrophage-activating factor of mammals (8, 9).

In contrast to mammals, the IFNs of birds are poorly characterized. The first cDNA for a chicken IFN was isolated only recently (10). Its sequence similarity to mammalian IFNs is marginal, but conservation of cysteine residues and inducibility by virus indicate that it represents a type I IFN. This notion was supported by the finding that recombinant chicken IFN is a potent antiviral agent that lacks other biological activities associated with IFN-γ of mammals (11). Antibodies to the cloned chicken IFN neutralized the bulk of antiviral activity in preparations of partially purified chicken IFN from various natural sources (11), suggesting that a single serotype of IFN is predominately induced under experimental conditions. These results were compatible with the assumption that the chicken has a single gene for type I IFN (10). However, Southern blot analysis now suggested the presence of several IFN genes in chicken. A more detailed analysis showed that the chicken genome contains a family of at least 10 IFN genes, now designated IFN1, which all appear to code for one serotype of chicken IFN. A second serotype of cIFN is encoded by a single gene, designated IFN2, that shows limited sequence conservation.

MATERIALS AND METHODS

Phage Library Screening—Approximately 1.2 × 10^10 plaques (10^9/mg) of a chicken genomic library constructed in λ FIX II (Stratagene) were screened with the previously cloned ChIFN cDNA (10) that was radiolabeled by nick translation. The membranes were hybridized at 42 °C in a buffer containing 10 mM PIPES, pH 6.8, 300 mM NaCl, 10 mM EDTA, 0.5% SDS, 1 × Denhardt's solution, 100 μg/ml denatured herring sperm DNA, and 20% formamide for 15 h before they were washed in 2 × SSC containing 0.5% SDS for 1 h at 42 °C and then for 30 min at 56 °C. Selected positive phages were plaque-purified three times.

Genomic Southern Blot Analysis—Samples (20 μg) of chicken liver DNA (a kind gift of Dr. B. Kaspers) were digested with 80 units of restriction enzyme, fragments were size-fractionated by electrophoresis through a 1% agarose gel, soaked in 0.2 M HCl for 10 min, and transferred to nylon membranes in 0.4 N NaOH. Radiolabeled DNA fragments comprising the complete coding regions of IFN1 or IFN2 were used as hybridization probes. Hybridizations were carried out at 42 °C in 0.12 M sodium phosphate, pH 7.3, 0.25 mM NaCl, 7% SDS, 1 mM EDTA, 20% formamide, and 200 μg/ml denatured herring sperm DNA. Linearized plasmids carrying the coding regions of IFN1 or IFN2 were subjected to 3-fold dilutions in buffer containing carrier DNA, and

*This work was supported by a grant from the Deutsche Forschungsgemeinschaft. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Dept. of Virology, University of Freiburg, Hermann-Herder-Strasse 11, D-79008 Freiburg, Germany. Tel.: 49-761-203-6579; Fax: 49-761-203-6562; E-mail: staeheli@sun1.ukl.uni-freiburg.de.

1 The abbreviations used are: IFN, interferon; ChIFN, chicken IFN; PIPES, 1,4-piperazinediethanesulfonic acid; kb, kilobase pair(s); PCR, polymerase chain reaction; VSV, vesicular stomatitis virus.
AFamily of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.

RESULTS

A Family of Genes Coding for Chicken IFN—To estimate the size of the IFN gene family, we performed genomic Southern blot analyses of liver DNA from blood group antigen-synthetic White Leghorn chicken. When the blots were hybridized to a radiolabeled probe derived from the previously cloned chicken IFN (ChIFN) cDNA (10), a simple pattern of strong and a complex pattern of weak hybridization signals was observed (Fig. 1A). By comparing the signal intensities to those of defined amounts of linearized plasmid DNA containing ChIFN cDNA (10), we calculated that 10 or more IFN genes were present in the chicken genome. Support in favor of this view came from the screening of a λ phage library of genomic chicken DNA: the ChIFN cDNA probe identified a total of 165 positive hybridization signals.

Virus YIELD Reduction Assays—These assays were performed with chicken CEC-32 cells infected with vesicular stomatitis virus (VSV) as described (13). Rabbit antiserum to purified recombinant ChIFN1 from E. coli was used at a dilution of 1:200 (13). Preimmune serum (1:200) from the same animal served as negative control.
IFN cDNA, again suggesting that the chicken IFN genes were intron-less. Partial sequencing of some PCR products showed that members of this gene family, which we now designate IFN1, had almost identical sequences.

To learn more about the IFN1 gene family, we decided to perform a more detailed analysis of one particular λ phage that yielded a very strong hybridization signal, suggesting that it contained more than one IFN1 gene. Restriction analysis and partial sequencing confirmed that this λ phage contained a 14.5-kb fragment of chicken DNA that harbored three intron-less genes, designated IFN1-1, IFN1-2, and IFN1-3 (Fig. 2).

Their sequences were almost identical to that of the previously cloned chicken IFN cDNA (10), except for an A to G transition at positions 202 and 227 in IFN1-1. The sequences of the three IFN1 genes were deposited with EMBL/GenBank™, accession numbers X92476, X92477, and X92478.

Recognition of a Novel Chicken IFN Gene—One λ phage showed particularly weak hybridization signals when probed with the ChIFN probe. Nucleotide sequencing of an EcoRI-BglII fragment of this λ phage revealed an intron-less gene, designated IFN2, whose coding sequence was 73% identical to the IFN1 genes (Fig. 3). To estimate the number of IFN2 genes in the chicken genome, we performed Southern blot analyses with a radiolabeled probe that comprised the complete coding region of the IFN2 gene. This probe cross-reacted weakly with the various DNA fragments that contained IFN1 genes. More importantly, it recognized additional fragments of approximately 13, 3, and 3.5 kb in chicken DNA digested with BamHI, HindIII, or PstI, respectively (Fig. 1B). By comparing the intensities of the IFN2-specific signals of the BamHI and HindIII digests with appropriate plasmid standards (Fig. 1B), we concluded that the chicken genome most likely contains a single IFN2 gene. We noted that the signal in the PstI digest was stronger than those in the neighboring lanes. Its increased intensity was probably artifactual due to a fortuitous superpositioning of the IFN2 signal with an IFN1 signal.

The IFN2 gene codes for a polypeptide of 203 amino acids (Fig. 3), whose N terminus lacks charged amino acids, suggesting that it may function as a signal peptide. An alignment of the ChIFN2 sequence with the prototype sequence of ChIFN1 (10) and the product of a recently cloned duck IFN gene (14) is shown in Fig. 4. This comparison indicated that the cysteine residue at position 28 is the N-terminal amino acid of mature ChIFN2. Secreted ChIFN2 thus seems to be 14 residues longer than ChIFN1: it is composed of 176 amino acids and has a calculated molecular mass of 20,372 Da. Sequence conservation between ChIFN1, ChIFN2, and duck IFN is pronounced in most regions, except for the signal peptides and the C termini. When the first 150 amino acids of the mature proteins are considered, ChIFN2 is 57% identical to ChIFN1 and 61% identical to duck IFN. The C-terminal 26 amino acids of ChIFN2 are identical to duck IFN. The C-terminal 26 amino acids of ChIFN2 are conserved in both chicken IFNs as well as in duck IFN.

Identification of a Novel Chicken IFN Gene—One λ phage showed particularly weak hybridization signals when probed with the ChIFN probe. No product was obtained when PCR was performed with DNA of this phage and IFN1-specific oligonucleotide primers, suggesting that it may contain a novel IFN gene. Sequence analysis of an EcoRI-BglII fragment of this λ phage revealed an intron-less gene, designated IFN2, whose coding sequence was 73% identical to the IFN1 genes (Fig. 3). To estimate the number of IFN2 genes in the chicken genome, we performed Southern blot analyses with a radiolabeled probe that comprised the complete coding region of the IFN2 gene. This probe cross-reacted weakly with the various DNA fragments that contained IFN1 genes. More importantly, it recognized additional fragments of approximately 13, 3, and 3.5 kb in chicken DNA digested with BamHI, HindIII, or PstI, respectively (Fig. 1B). By comparing the intensities of the IFN2-specific signals of the BamHI and HindIII digests with appropriate plasmid standards (Fig. 1B), we concluded that the chicken genome most likely contains a single IFN2 gene. We noted that the signal in the PstI digest was stronger than those in the neighboring lanes. Its increased intensity was probably artifactual due to a fortuitous superpositioning of the IFN2 signal with an IFN1 signal.

The IFN2 gene codes for a polypeptide of 203 amino acids (Fig. 3), whose N terminus lacks charged amino acids, suggesting that it may function as a signal peptide. An alignment of the ChIFN2 sequence with the prototype sequence of ChIFN1 (10) and the product of a recently cloned duck IFN gene (14) is shown in Fig. 4. This comparison indicated that the cysteine residue at position 28 is the N-terminal amino acid of mature ChIFN2. Secreted ChIFN2 thus seems to be 14 residues longer than ChIFN1: it is composed of 176 amino acids and has a calculated molecular mass of 20,372 Da. Sequence conservation between ChIFN1, ChIFN2, and duck IFN is pronounced in most regions, except for the signal peptides and the C termini. When the first 150 amino acids of the mature proteins are considered, ChIFN2 is 57% identical to ChIFN1 and 61% identical to duck IFN. The C-terminal 26 amino acids of ChIFN2 are identical to duck IFN. The C-terminal 26 amino acids of ChIFN2 are conserved in both chicken IFNs as well as in duck IFN.
rum, ChIFN1 as well as ChIFN2 were very effective and rabbi
t antiserum or preimmune serum, before the cultures
bated with 100 units/ml of the various IFNs and 0.5% of either
units/mg, which was about 20 times lower than that of ChFN1
which was purified in parallel.

to determine whether ChFN1 and ChFN2 represent two

different serotypes of chicken IFN, we evaluated the cross-
neutralizing potential of a rabbit antiserum that we had prepared
against E. coli-produced ChFN1 (13). CEC-32 cells were incu-
inated with 100 units/ml of the various IFNs and 0.5% of either
rabbit antiserum or preimmune serum, before the cultures
were challenged with VSV. In the presence of preimmune se-
rum, ChFN1 as well as ChFN2 were very effective and re-
duced the VSV yields by almost 10-fold (Fig. 5). In the pres-
ence of antiserum, the activity of ChFN1 was neutralized
quite effectively. By contrast, the antiserum did not signifi-
cantly reduce the antiviral activity of ChFN2 from E. coli or
form transfected COS7 cells (Fig. 5), suggesting that it repre-
sents a novel serotype of chicken IFN.

DISCUSSION

We have shown here that the chicken contains at least two
serologically distinct subtypes of IFN. The first subtype is
coded for by a gene family of more than 10 members, while the
second subtype seems to be encoded by a single gene. All these
genes lack introns, like the mammalian genes for the various
IFNs. Thus, although the primary structures of avian
IFNs are poorly conserved, the presence of a gene family rather
than a single gene and the lack of introns are highly conserved
features of type I IFNs.

The search for additional subfamilies of chicken IFNs is
complicated by the fact that the IFN1 gene family is quite
large. Southern blot analysis suggested the presence of about
10 IFN1 genes. However, the high frequency by which positive
λ phages were identified in a chicken genomic library sug-
ggested the presence of as many as 100 IFN genes in the chicken
genome. It is possible that the latter high frequency is an
artifact of the phage library. Alternatively, the library screen
may indeed have revealed the true complexity of the chicken
IFN superfamily. We assumed that the complex pattern of
weak hybridization signals on the Southern blot (Fig. 1A) re-
sulted from single IFN1 genes with altered restriction sites in
their flanking regions. However, we cannot exclude the alter-
native possibility that these signals resulted from weak cross-
reactivity to a novel IFN gene family.

One cross-reactive λ phage that we characterized contained a
novel chicken IFN gene that we designated IFN2. Southern
blotting experiments suggested that it is a single copy gene.
Functional studies showed that IFN2 codes for a chicken IFN
that escapes neutralization by antibodies to ChFN1, a finding
that may be explained by the fact that the amino acid sequence
of ChFN2 is only 57% identical to that of ChFN1. Interest-
ingly, mature ChFN2 is 14 amino acids longer than ChFN1.
In mammals, “long” variants of α-IFN with extra amino acids
at the C terminus are known as α- and γ-IFNs (2). Their
sequences are about 60% identical to α-IFNs, and their activi-
ty cannot be neutralized by antisera that neutralize α-IFNs
(5). Although this suggests that ChFN2 represents the avian
homolog of mammalian α- or γ-IFN, we believe that the familiar
terms α-, β-, ω-, and γ-IFN, which refer to subtypes of
mammalian IFNs with specific biological properties, should not
be used at present for the chicken IFN system. Detailed studies
on the induction of IFN2 in response to virus or developmental
stimuli will be required to determine whether this assumption
is correct.

An unexpected result of our studies was that the specific
antiviral activity of purified recombinant ChFN2 was about 20
times lower than that of ChFN1. Evidence that this difference
was not an artifact of the purification procedure came from
experiments with supernatants of COS7 cells that were trans-
fected with expression constructs for ChFN1 or ChFN2. Be-
cause the two constructs were identical except for the IFN
coding regions, it seems reasonable to assume that similar
amounts of recombinant protein were produced in the two
cultures. Nonetheless, the supernatants of ChFN1-producing
cultures contained about 20-fold more antiviral activity than
supernatants of ChFN2-producing cultures, strongly suggest-
ing that this difference reflects a true biological difference of
the two IFN2 subtypes. This result further indicated that the
low specific activity of E. coli-produced ChFN2 cannot be ex-
plained by simply assuming that ChFN2 needs glycosylation
for full activity. This situation found here for ChFNs is remi-
niscent to that described for the various α-IFN subtypes of
humans and mice, whose individual specific activities were also
found to differ significantly (15, 16).

REFERENCES
1. Pestka, S., Langer, J. A., Zoon, K. C., and Samuel, C. E. (1987) Annu. Rev.
Biochem. 56, 727–777
2. De Mayer, E. M., and De Mayer-Guinard, J. (1988) Interferons and Other
Regulatory Cytokines, Wiley Interscience, New York

3. Imakawa, K., Anthony, R. V., Kazemi, M., Marotti, K. R., Polites, H. G., and Roberts, R. M. (1987) Nature 330, 377–379

4. Whaley, A. E., Meka, C. S., Harbison, L. A., Hunt, J. S., and Imakawa, K. (1994) J. Biol. Chem. 269, 10864–10868

5. Addf, G. R. (1987) J. Gen. Virol. 68, 1669–1676

6. Dijkmans, R., and Billiau, A. (1988) Curr. Opin. Immunol. 1, 269–274

7. Ijzermans, J. N., and Marquet, R. L. (1989) Immunobiology 179, 456–473

8. Pace, J. L., Russell, S. W., Schreiber, R. D., Altman, A., and Katz, D. H. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 3782–3786

9. Dijkmans, R., Creemers, J., and Billiau, A. (1990) Vet. Immunol. Immunopathol. 26, 319–332

10. Sekellick, M. J., Ferrandino, A. F., Hopkins, D. A., and Marcus, P. I. (1994) J. Interferon Res. 14, 71–79

11. Schultz, U., Kaspers, B., Rinderle, C., Sekellick, M. J., Marcus, P. I., and Staeheli, P. (1995) Eur. J. Immunol. 25, 847–851

12. Burt, D. W., Burnstead, N., Bitgood, J. J., Ponce-de-Leon, F. A., and Crittenden, L. B. (1995) Trends Genet. 11, 190–194

13. Schultz, U., Rinderle, C., Sekellick, M. J., Marcus, P. I., and Staeheli, P. (1995) Eur. J. Biochem. 229, 73–76

14. Schultz, U., Köck, J., Schlicht, H. J., and Staeheli, P. (1995) Virology 212, 641–649

15. Shaw, G. D., Boll, W., Taira, H., Mantei, N., Lengyel, P., and Weissmann, C. (1983) Nucleic Acids Res. 11, 555–573

16. Weissmann, C., and Weber, H. (1986) Prog. Nucleic Acids Res. Mol. Biol. 33, 251–300
