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NETHERLANDS MEETING

# THE BIOLOGY OF THE INTERFERON SYSTEM

The Netherlands Organization for Applied Scientific Research (TNO) sponsored its second conference on "The Biology of the Interferon System" on 18–22 April 1983 in Rotterdam. Although it came only six months after the Third International Congress for Interferon Research, the meeting was both successful and informative. An unannounced item on the agenda was the election of a committee to review and revise the bylaws of the International Society for Interferon Research (ISIR), a new scientific association. One important task for ISIR will be to encourage better coordination between European and American researchers and limitation of meetings to one large symposium each year. Too much of a good thing may become a bad thing; obtaining scientific results requires some time to work at the bench.

Interferon (IFN) is, in many ways, an exemplary biotechnology product. Its discovery 25 years ago spawned great hopes for a major breakthrough in human therapeutics and considerable profits for interested industries. A major hurdle for industrial development, the limited availability of pure material, has largely been overcome in the past few years. K. Cantell developed a convenient method for production of human interferons from buffy coats, a component of blood, that permitted scaling up of production to the limit of the availability of blood donated in Finnish transfusion centers. In 1980, C. Weissman and his collaborators first achieved expression of an interferon gene in *E. coli*. These developments stimulated the interferon field and marked the beginning of the industrial era of the interferon system. (The expression "the interferon system" alludes to the multiple potentialities of interferon. Coined in 1979, it refers to the fact that different classes of interferon exist that exert different biological activities via a number of different pathways.)

Much more research is needed to exploit efficiently all the activities of the interferon system, and most of the presentations at the Rotterdam meeting focused on development of interferon technology rather than its specific applications. For example, much of the current research on interferon requires large quantities of pure, well-characterized protein; thus the cloning and expression of the different genes coding for interferons are essential for progress. Studies of the molecular structure of the proteins in relation to their biological properties will provide information about their mechanism of action, and how they can be exploited more efficiently. Animal studies will be necessary to establish rational protocols for further clinical trials; appropriate animal models will permit researchers to characterize the interferon actions that are useful for therapeutics and to screen for possible toxic effects.

**A Belgian research group treated this calf with human $\alpha_2$ interferon to protect it against an experimental virus infection. The interferon was provided by C. Weissman.**

### Cloning and Expression of Interferon Genes

W. Fiers (University of Ghent, Belgium) reviewed the work of his group on cloning the $\beta$ and $\gamma$ interferons. They also utilized a SV40 vector system and obtained expression of $\beta$ IFN in monkey cells. The $\gamma$ IFN gene contains three introns, three potential Asn glycosylation sites, and a very long 3′ untranslated sequence. Fiers also reported that his group has cloned the gene for interleukin 2, as has Taniguchi[1]. This immune modulator is required for full activation of natural killer (NK) cells by $\gamma$ interferon.

R. Derynck (Genentech, U.S.) described his group's work on cloning the human $\gamma$ IFN gene in *E. coli* and yeast, and described some new results on cloning $\gamma$ IFN genes from mice and cows. He noted that the transformed bacteria that express IFN contain a large refractile body; interferon is associated with this structure, and its existence may point the way for designing a better method for recovery of interferon from these cells.

Creasey (Cetus Corp., U.S.) has characterized the biological activity of four novel recombinant human $\alpha$ IFNs and compared it to $\alpha_1$ IFN. There were no significant differences in their ability to induce 2′5′ adenylate (A) synthetase, increase HLA expression, or their activity in the clonogenic assay using melanoma cells. However, one of the IFN clones appeared to activate NK cells more

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efficiently, and the different clones had different effects on DAUDI cell growth and enhancement of protein kinase activity.

These results suggest that structure-function relationship studies may provide information about the different domains of the interferon molecule that are important for its different biological activities. K. Alton described the strategy his group at AMGen (U.S.) is pursuing. They synthesized α and γ IFN genes, cloned them in *E. coli,* then produced analogs with either single or multiple amino acid substitutions. For example, they constructed a consensus α IFN type with minor substitutions in the non-conserved region of the protein, then tested the antiviral activity of three of these variants against vesicular stomatitis virus and encephalomyocarditis virus. They found relative differences in the activities of the different variants.

J. Collins (Braunschweig, F.R.G.) is using a library of human placental DNA to detect other heterogeneities in interferon genes. His results add three more species to the 14 α IFN subtypes already identified.

Independent work from the laboratories of P. Pitha (Johns Hopkins University, U.S.) and D. Burke (formerly of Warwick University, U.K., now at Allelix, Canada) indicates that Sendai virus induces transcription of both α and β IFN genes in Namalwa cells. β IFN is not produced in Namalwa cells; thus induction must be regulated both at the transcriptional and post-transcriptional level. Pitha analyzed transcripts in isolated nuclei and found high molecular weight transcripts of the 3′ regions downstream from the β IFN coding regions. J. Content (Institut Pasteur, Belgium) has obtained similar results and characterized the RNA transcribed in osteosarcoma cells. Upon induction with poly I-C, both an 11S mRNA species and a 3.8 kb poly A-containing transcript are made. However, the physiological significance of this large transcript, which starts at the cap site, is unclear.

### Molecular Structure of Interferon

Genetic manipulation is beginning to provide useful information about the relationship between the molecular structure of IFN and its specific biological activities. D. Mark (Cetus Corporation, U.S.) used a specific mutation approach to construct a β IFN variant lacking one of the three cys residues. The variant is a very stable molecule with increased biological activity. R. Wetzel (Genentech, U.S.) reported that cystine bridges are important either for the correct folding of the β IFN molecule or for its activity. Artificial hybrid interferons, as well as natural recombinants, have different patterns of antiviral, anticellular, and NK cell activation activities, according to S. Pestka (Roche Institute, U.S.). The relationship between anticellular and antiviral activity in the hybrids varied enormously, leading Pestka to conclude that IFN action cannot be interpreted simply in terms of a receptor-ligand interaction that triggers a specific event.

A complex model for IFN action is also consistent with an accelerated electron target analysis of the protein molecule. By following the course of IFN inactivation with an accelerated electron beam, Pestka was able to deduce the size and molecular weight of the active structure. The data indicated that α IFN is active as a monomer, β IFN is active as a dimer, and γ IFN is active as a tri- or tetramer.

### Biological Activities

M. Aguet (Zurich University, Switzerland) has been studying the cell-binding properties of six different subclasses of human α IFN. They all compete for a common binding site, and there are $5 \cdot 10^2$–$5 \cdot 10^3$ high affinity binding sites per cell. The affinity constant varies from $10^{-12}$ to $10^{-10}$ M and the binding affinity correlates well with antiviral activity. Aguet noted that the bound IFN molecules are internalized and degraded, but it is not known whether these processes are important for biological activity.

Specific receptors for human $\alpha_2$ IFN have been identified using cross-linking agents. S. Gupta (Memorial Sloan-Kettering, U.S.) used $^{125}$I-labelled solid phase lactoperoxidase to cross link the receptor-IFN complex on human cells. Polyacrylamide gel electrophoresis analysis revealed a 150 kilodalton complex; the binding protein is probably a glycoprotein and the reaction does not involve gangliosides.

### The Premier Lymphokine

The striking immunoregulatory functions of γ IFN inspired J. Vilcek (New York University, U.S.) to describe it as the "premier lymphokine." Y. Kawade (Institute for Virus Research, Kyoto) described work on a clone of a T-cell line grown in the presence of rat T-cell growth factor. Upon conA stimulation these cells produced $10^3$ to $10^4$ U/ml IFN in serum-free medium. Polyacrylamide gel electrophoresis resolved two components of interferon activity, molecular weights 35 and 22 kilodaltons. If the cells are induced in the presence of tunicamycin, an inhibitor of glycosylation, a new 18 kilodalton component is observed. This is the size predicted for an unglycosylated polypeptide on the basis of nucleotide sequences.

### Mechanism of Action of Interferon

Reviewing the present knowledge of the mechanism of action of interferon, I. Kerr (ICRF, U.K.) stressed that α, β, and γ IFN all have the same fundamental effects. However, there are several important quantitative, and sometimes qualitative, differences. Even the role of 2′5′ A synthetase, which has been shown to be important for interferon activity in some virus-cell systems, is not general. Moreover, the presence of 2′5′ A synthetase appears to be of wider significance since increased levels have been detected in hen oviduct after withdrawal of estrogens[2] and its activity is modulated in regenerating rat liver after a partial hepatectomy (M. Smekens-Etienne, Université Libre de Bruxelles, Belgium). A. Kimchi (Weizmann Institute, Israel) reported that an interferon produced during the process of Friend cell differentiation induces 2′5′ A synthetase. However, E. Affabris (Instituto Superior di Sanita, Italy) showed that a variant line of Friend cells that is resistant to α and β IFN develops an antiviral state upon treatment with γ IFN without production of any detectable 2′5′ A synthetase activity.

It is clear that multiple pathways are involved in the mechanism of action of IFN. J. Taylor-Papadimitriou (ICRF, U.K.) summarized the known biological effects of IFN that are probably mediated via induction of specific proteins. The pattern of these proteins depends on the type of IFN and the kind of cell used, according to L. Epstein (University of California, San Francisco, U.S.). M. Horisberger (CIBA-GEIGY, Switzerland) described one protein that is associated with interferon-induced resistance to influenza virus infection in certain mouse strains. Treatment with either α or β IFN confers resistance, while treatment with γ IFN does not. A protein with a more general action is the RNAse described by R. Silverman (NIH, U.S.), whose pattern of induction is parallel to the IFN sensitivity of the cells studied.

M. Revel's group (Weizmann Institute, Israel) isolated clones of some of the induced proteins. They obtained clones of 2′5′ A synthetase and for a 56 kilodalton protein of unknown function that is detected very early after IFN treatment. P. Lengyel (Yale University, U.S.) demonstrated that 2′5′ A synthetase is heterogeneous: different enzymes are present in the cytoplasm and nucleus, which are coded for by different mRNAs.

M. F. Dubois (INSERM, France) showed that IFN prevents stable integration and expression of exogenous genes during transfection. This effect

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was also observed by B. Friedman's group (NIH, U.S.) using the *ras* oncogene. These results on cultured systems suggest that IFN may play a role in the regulation and preservation of established cellular genes. Finally, P. Whitaker-Dowling observed that IFN inhibits virus infection in cells by preventing viral entry. This effect may be the basis for the ability of IFN to inhibit the multiplication of intracellular parasites.

**Interferon Monoclonal Antibodies**

Monoclonal antibodies against IFN are providing better and more efficient reagents for purification processes. In addition to the NK2 monoclonal antibody commercialized by Celltech (U.K.), several other antibodies against α IFN have recently been developed. D. Secher (MRC Cambridge, U.K.) described a new antibody that recognizes a different spectrum of human α IFN subtypes than the NK2 products. When the two antibodies are used together, about 90 percent of α IFN activity can be recovered from crude leukocyte IFN preparations. This permits a high degree of purification in a single step.

K. Berg (Aarhus University, Denmark) described the properties of another monoclonal antibody, designated LO-22, in the poster session. It binds more than 95 percent of human leukocyte IFN activity and has been used to purify recombinant α IFN. This antibody appears to recognize a common antigenic determinant present on all species of α IFN.

**Animal Studies with Interferon**

H. Schellekens (Primate Center, TNA, Netherlands) stated clearly that successful clinical application of IFN will require good animal studies. Many of the animal studies have involved IFN treatment in conjunction with other chemotherapeutic agents. Chany (INSERM, France) reported that sequential treatment of mice with adriamycin and IFN results in a striking enhancement of the toxicity of adriamycin. This may shed some light on recent French clinical studies in which three of the four patients who died during IFN treatment had previously received adriamycin.

Animal models are also useful for approaching the study of the diverse *in vivo* properties of the interferon system. H. Kirchner (Heidelberg, F.R.G.) observed that some mouse strains have interferon-mediated resistance to herpes simplex virus. E. De Maeyer (Institut Curie, France) discovered that the Lou-C strain of rat, which has a high incidence of myeloma, spontaneously produces anti-IFN antibodies. The level of these antibodies increases with age and this system may be an excellent model for studying the consequences of anti-IFN, antibody production during autitumor therapy, a clinical situation that is sometimes encountered.

A new model system for studying the effects of human $\alpha_2$ IFN has been developed by the author, J. Werenne (Université Libre de Bruxelles, Belgium), which has provided the first evidence for *in vivo* antiviral IFN activity in bovine species. Human $\alpha_2$ IFN could fully protect calves against an experimental viral infection. The effective dose was $10^6$ U/kg, although there was some individual variation in sensitivity. Werenne's group has also shown that endogenous IFN plays a role in control of rotavirus infection in newborn calves. These data point to a new role for IFN in veterinary therapeutics.

**Clinical Studies**

Studies on patients with AIDS or SLE reveal that an acid-labile α IFN accumulates in the circulation and elevates 2′5′ A synthetase activity in individuals with these immunological disorders (O. Preble, NIH, U.S.). Most clinical studies are still underway, but evidence is accumulating that IFN plays an important role in many pathological situations. S. Yamazaki (NIH, Japan) summarized the current status of clinical research in Japan. The most promising results have been obtained with tumors of probable viral origin such as skin warts. M. Ng (University of Hong Kong) also reported encouraging results for local IFN therapy of cervix dysplasia.

Cloned IFN protects human volunteers against infection by rhinovirus and coronavirus, but it induces uncomfortable side effects that resemble the natural symptoms of the common cold (G. Scott, MRC Salisbury, U.K.). One is left with the impression that pure individual IFNs are not as effective as their endogenous counterparts. It appears that leukocyte IFN prepared according to Cantell's method may be more active. It is not known at present whether this is due to synergistic interactions between the different IFN subspecies or to a cooperative factor that is present in the leukocyte IFN preparation.

If biotechnology is going to produce clinically useful interferons, more research must be done. The different subspecies must be defined and produced in large quantities. Better biochemical markers and molecular probes must be developed so that the *in vivo* interferon response can be dissected and understood. Only then can researchers develop technologies for mimicking and enhancing these responses. Clearly there is an interesting future for interferon research and development.


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**CANDICIDIN PRODUCTION**

# CLONING OF A GENE INVOLVED IN ANTIBIOTIC SYNTHESIS

Microorganisms of the genus *Streptomyces* are the main producers of antibiotics and other secondary metabolites. Despite their industrial importance the molecular biology of Streptomyces has been studied very little. Even more surprising is the fact that the biosynthetic pathways of many important antibiotics are only partially known. For example, knowledge of the enzymology of the biosynthetic steps of β-lactams (penicillins, cephalosporins, cephamycins) is very scanty. The regulatory mechanisms that control antibiotic biosynthesis, which must be bypassed to get high production of these compounds, are only superficially understood[1,2].

This situation seems to be changing, although at a slow pace. The recent development of DNA cloning techniques applicable to Streptomyces has advanced the study of the molecular genetics of these organisms[3–5]. Promoter-probe plasmid vectors have been constructed in *Streptomyces lividans* and used to study the activity of DNA sequences that contain transcriptional control signals[6]. Several different laboratories are now using these vectors to develop a detailed picture of the expression of genes involved in antibiotic synthesis, differentiation, and other biological