# Interferons

# 7

**Abstract**

Interferon was discovered by Alick Isaacs and Jean Lindenmann in 1957. It was originally thought that interferon could be used as a general anti-viral agent and in anti-cancer therapy. There are many different types of interferons, now known as interferons "alpha," "beta," "gamma" and "lambda," with different cellular receptors and modes of action, and there are possibly 24 different types of alpha interferon. Independently and simultaneously, a group of Japanese scientists found an "interfering factor," which upon subsequent analysis turned out to be interferon, probably of the alpha type. The interferon alpha gene was the first mammalian gene to be cloned in a bacterial system and became the prototype for gene cloning technology. Until the cloning of the interferons in *Escherichia coli*, and expression of the interferon genes in mammalian cells in culture, it was impossible to obtain enough material for clinical use. Interferon today is predominantly used in the treatment of hepatitis B and C, leukemia and Kaposi's sarcoma. As an anti-viral agent, interferon has not lived up to its initial promise, since in vitro most viruses block the activity of interferon and clinical trials have given inconclusive results with severe side effects. Interferon induces hundreds of genes in vivo and in vitro, each interferon producing overlapping and distinct gene profiles. The mechanism of both interferon induction and anti-viral response is complicated and involves the interaction of many regulatory molecules. Interferon is now known to be a component of the large family of cytokines or interleukins.

M. W. Taylor, *Viruses and Man: A History of Interactions*,
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## 7.1 Discovery of Interferon

No history of virology would be complete without a discussion of interferons and how they led to the discovery and identification of cytokines (small proteins that influence the activity of the immune system and nearby cells), their function in innate immunity, and their pharmaceutical properties as anti-viral and anti-cancer agents. The cloning of the interferon gene and its production in *E. coli* initiated the biotechnology revolution. As was the case of many other major discoveries in science, interferon was a fortuitous discovery.

In 1957, Alick Isaacs (1921–1965) and a post-doctoral Swiss student, Jean Lindenmann, were studying the phenomenon of "viral interference"—the ability of one virus to inhibit the replication of another virus. When 10-day-old chick chorioallontoic membranes from chick embryos were infected with heat or UV inactivated influenza virus, a material was produced that interfered with subsequent viral replication. The experimental procedure is illustrated in Fig. 7.1. Influenza virus production (or inhibition) was measured by hemagglutination, the ability of the virus to interact and agglutinate red blood cells. They termed the interfering substance "interferon".The end point of the titration was the identification of that well (on a plate of small wells) with partial agglutination; the reciprocal of the influenza dilution thus observed was taken as the interferon titer (concentration).

The first reports of interferon were published in 1957 in the prestigious *Proceedings of the Royal Society*, [1, 2] and a more detailed description in the *British Journal of Pathology* [3]. Using standard biochemical techniques, it was shown

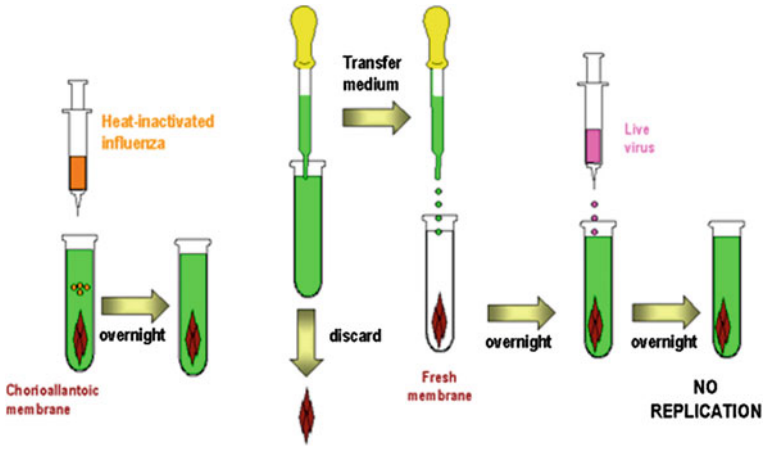


**Fig. 7.1** The Discovery of intereferon. From Isaacs and Lindenmann, Proc. Roy Soc B, 1957 (adapted from http://www.microbiologybook.org/book/virol-sta.htm)

that interferon was a protein, was pH 2 resistant, could be precipitated by ammonium sulfate, destroyed by ether, and digested by trypsin—all characteristics of proteins. The stability at pH 2 was an important characteristic, later used to distinguish the original interferon (called IFN-alpha) from other later discovered interferons. Attempts to initially purify the interferon were unsuccessful. A very low concentration was induced and the investigators at that time were unaware that multiple types of interferon existed, so that even with the techniques of column chromatography available at that time, it proved impossible to purify a single type.

Jean Lindenmann returned to Zurich and did not continue to work on interferon but continued research on influenza virus, discovering a strain of mice resistant to the virus. This resistance did not appear to be related to interferon, but to the Mx protein coded by an autosomal gene, *MX*. However, in later years Lindenmann and colleagues found that the Mx protein was interferon-inducible, and that this protein was a component of the cascade of genes induced by interferon.

Rather surprisingly, interferon was not viral-specific; it not only inhibited influenza, the inducing virus, but also unrelated vaccinia and other viruses [4]. That interferon was an agent with a very wide inhibitory range suggested that interferon could be used as a general anti-viral agent, much as the recently discovered antibiotics. As a result, three pharmaceutical companies, Glaxo Laboratories, ICI Pharmaceuticals, and Burroughs Wellcome (later to become the Wellcome Foundation) supported the research. The primary purpose was to produce enough interferon for use in clinical trials, and a second was to keep the patent for interferon production in the U.K., since there was the feeling that penicillin, a British discovery, had been appropriated by the U.S. (See Burke [5] for a review of the early days of interferon research.)

## 7.2 Inhibitory Factor

In parallel with the research described above, but quite independently, in 1954 a group of researchers in Japan characterized what they called a "virus inhibitory factor" [6]. They injected ultra-violet irradiated vaccinia (inactivated virus) into the backs of rabbits and subsequently inoculated them with live vaccinia at various times after the initial inoculation. The vaccinia replication was inhibited by the pre-treatment with the inactivated virus. They isolated this substance and called it "inhibitory factor" (IF). Further experimentation was difficult because of the system used. These observations were presented at the 1956 Annual Meeting of the Japan Society for Viral Research and later at the meeting of the Japan-France Biology Society held in 1957. This was really the first report on interferon but was not recognized as such until much later. The IF was later tested for interferon activity using rabbit standard interferon, designated as such by the International Committee on Interferons, and it was found to have an interferon titer of 300,000 IU, an astonishingly high level of activity. It is now clear that Yasuichi Nagano and Yasuhiko Kojima observed the production of interferon in response to

an inactivated DNA virus in the whole animal. Because this work was carried out in Japan, and originally published in a French journal, it was not given the recognition it deserved. (A fuller description of this study can be found in the review by Ozato and colleagues [7].)

## 7.3 First Clinical Trials

An ambitious first "clinical trial" was performed at the Salisbury Common Cold Center in England in 1962. Its purpose was to examine whether interferon inhibited the production of vaccinia virus following injection of the virus into the arms of individuals who had not been previously vaccinated with vaccinia. The result was limited inhibition of vaccinia replication; it was also obvious that much more interferon was needed if it was to be of clinical value, but it was not until the 1980s that enough interferon could be produced in a pure state for this purpose. Prior to gene cloning, it was extremely difficult to produce enough interferon for clinical use, even when attempting to produce it in cells in culture. In the 1960s there was a large demand for interferon in order to test it as an anti-cancer drug. Kari Cantell in Finland produced leukocyte interferon, in large vats, in quantities sufficient for some limited clinical trials. He harvested leukocytes from many sources and infected them with the Sendai virus, which greatly stimulated the production of interferon. However, even with these facilities, it was impossible to produce quantities sufficient for all the desired clinical trials (Fig. 7.2).

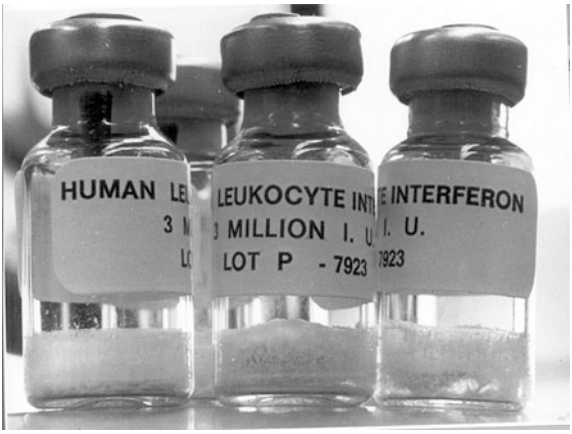


**Fig. 7.2** Vials of leukocyte interferon

## 7.4 How Does Interferon Protect Cells Against Virus Infection?

During the 1960s, many different viruses were shown to induce interferon in cell culture, and interferon in turn—when added to cells in culture—could inhibit most viruses, particularly at high concentrations, but not all, since it was realized much later that some viruses had developed methods of neutralizing interferon activity. Interferon was measured by its anti-viral activity, one unit of interferon being sufficient to inhibit viral growth by 50 %, usually measured by a plaque assay. Since there was some variability depending on the cell line and virus used, standard lots were titered or measured the NIH and supplied as "standards" to laboratories on request. When interferon was added directly to virus, there was no effect; it was the addition of interferon to the cells that inhibited virus production. A virus-infected cell produces interferon, which protects nearby cells; thus. the nearby cells are in an anti-viral state. (This is illustrated in Fig. 7.3).

Interferons are not only induced by viruses, but by viral intermediates such as double-stranded RNA, by synthetic double-stranded RNA such as PolyI:PolyC, some species of bacteria, endotoxins, and other cytokines. By the 1970s, a number of different types of interferon were characterized. Differences were found on the basis of cell types protected, and the stability of the interferons at different pH (Table 7.1). The binding of interferon to specific receptors on the cell triggers a large number of biochemical reactions, leading to the inhibition of virus replication or maturation and the induction of further interferon and other cytokines. The system is much more complex than the early researchers of this field could ever have imagined.

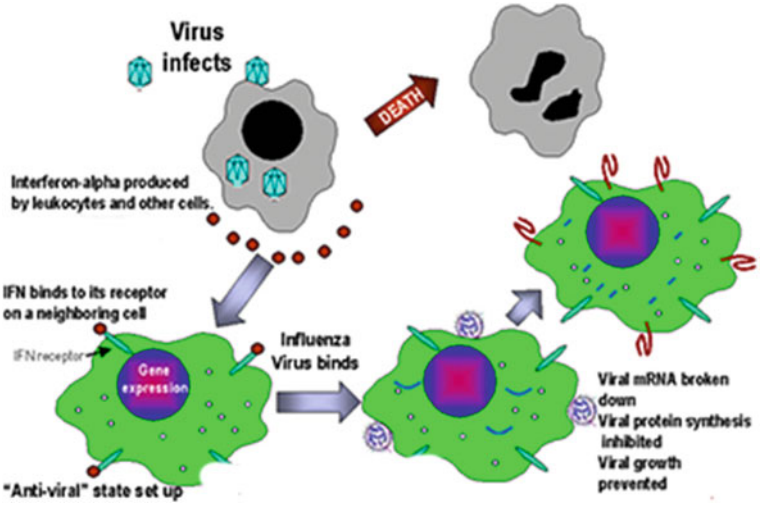


**Fig. 7.3** How interferon affects neighboring cells. Adapted from Hunt (http://www.microbiologybook.org/book/virol-sta.htm)

**Table 7.1** Classification and characteristics of human interferons

| Characteristics | Interferon-alpha | Interferon-beta | Interferon-gamma | Interferon-lambda |
|---|---|---|---|---|
| Other designation | Intron-A, pegasys. consensus | IFN-b2. At one time mistakenly called IL-6 | Macrophage activating factor: Immune-interferon | IL28A, IL28 B IL29, IFNA14 |
| Number of genes | 24 (+)? | 1 | 1 | 3 (+) |
| Chromosomal location | 9p22 | 9p21 | 12q14 | 19q13.13 |
| Introns in gene | None | None | Yes | Possibly yes |
| Cell of origin | Leukocytes | Fibroblasts | Lymphocytes, macrophages, NK cells, dendritic cells | Epithelial cells |
| Inducers | Virus, dsRNA | Virus, dsRNA | Antigens, mitogens, other interferons, cytokines, IL2, NK receptors | Virus |

Using mice as a model, experiments were initiated to examine whether interferon produced in mouse cell culture culture could be used to inhibit virus infection in mice, If given in large daily doses, interferon did inhibit the pathological effects of the EMC virus, a virus causing paralysis and death in mice [8]. Antibodies were made to mouse and human interferon in rabbits that neutralized the anti-viral effect in the mouse and the anti-viral effect of human interferon-alpha.

## 7.5 Classification of Interferons

It was rather obvious by the 1970s that there were at least three major types of interferons: alpha, beta, and gamma. These were defined as interferons because of their anti-viral activity in vitro. It was shown in 1984 [9] that there were separate receptors for IFN-gamma and interferon alpha/beta [10]. IFN-alpha and -beta attached to the same receptor(s) on the cell membrane, but the biological activity of each of these three interferons was different.

Table 7.1 summarizes the differences between class I interferons (IFN-alpha and beta) and class II interferon, IFN-gamma. The third column presents what is known of a recently discovered interferon, IFN-lambda [11, 12].

## 7.6 Cloning of Interferon Genes

In 1980 the interferon alpha gene was cloned into *E. coli* and the methodology used became standard for cloning mammalian genes in this bacterium. Double-stranded complementary DNA (cDNA) was prepared from random mRNA from

interferon-producing leukocytes (stimulated with Sendai virus) and cloned into an *E. coli* plasmid, known as pBR 322. Five thousand bacterial clones were screened by extracting plasmid DNA; they were expressed in frog oocytes, and those synthesizing biologically active interferon isolated. mRNA coding for a polypeptide with IFN activity was isolated, and the IFN was shown to have normal antiviral activity [13]. This research was done in the laboratory of Charles Weissmann at the University of Zurich, and supported by a new biotech company, Biogen. Fifteen percent of the shares of Biogen were held by Schering-Plough, which eventually sold the interferon as Intron A in the U.S. market. This major breakthrough in the cloning of IFN-α2a led to the cloning of other interferons. Meanwhile, other biotech and pharmaceutical companies entered into this very competitive market. The Wellcome Research Laboratories, one of the initial companies involved in interferon research in the U.K., extracted interferon from virus-transformed lymphoblastoid cells, and Searle Laboratories, also in England, produced interferon from fibroblasts. Biogen went on to sell interferon-beta 1a (Avonex) for the treatment of multiple sclerosis, and eventually joined up with a company specializing in monoclonal antibodies, Idec, to form one of the largest biotech companies in the world. Interpharm in Israel began the manufacture and production of IFN-beta in Chinese hamster ovary cells in culture, and finally interferon gamma was cloned in 1981 at the Genentech Company in San Francisco. Thus interferon could now be produced in *E. coli,* yeast, and mammalian cells [14, 15]. Interferon cloning led to the development of the biotech industry, and for this alone it is historically significant.

## 7.7 Interferon Alpha and Beta

Class I interferons have been studied more than class II, reflecting the fact that they were discovered first, cloned early, and used in the clinic. There appear to be many types of IFN-alpha based on gene homology, [16] but only one type of IFN-beta. Since the genes for all class 1 interferons reside on the same chromosome, it is assumed that they arose as duplications of a single interferon gene. Using high performance (or pressure) liquid chromatography (HPLC), it became possible to purify the various interferons and assay them with a biological assay. Using fluorescent tags, the amino acid content and sequence of each type of interferon was determined. There are as many as 24 different interferons that are classified as IFN-α. Each one has a slightly different amino acid sequence, different specific activity and antiviral spectrum [16]. Different cell types make different amounts of each type; different genes codes for each one. Very little is known of the biological bases of the multiple species and their activities, since most research has only been done with one of these, termed IFNα2. This may not be the most active of the alpha interferons; all the alpha interferons bind to the same receptor, and when one looks at gene (protein) induction as measured by a few key induced proteins, the same level of induction occurs in all cases, suggesting that a minimal amount of interferon triggers activity [16]. Interferon-alpha is induced in various types of leukocytes.

There is only one known species of IFN-beta, which shares 25 % amino acid homology with IFN-α. IFN-$\beta$ is produced by fibroblasts and epithelial cells in response to virus and double-stranded RNA.

In the early 1990s a group of scientists at the Amgen biotech company attempted to go one better than nature and create what they termed a "consensus interferon," which was made by comparing the most common amino acids occurring in the then-available 20 different species of IFN-alpha, constructing a " gene" from these amino-acids introducing this new "gene" into *E. coli*, and producing an active molecule. A number of such molecules were produced, one of which had very high specific activity. This molecule had higher specific activity in anti-viral activity, and anti-tumor activity, as measured by the death of hairy cell leukemia cells, and the activation of natural killer cells [17, 18]. Eventually, consensus interferon was approved for the treatment of hepatitis C. A well-planned clinical trial was never performed to examine whether consensus interferon was superior for the treatment of hepatitis C than IFN-α2a (intron A) or IFN-α2b (Pegasys). However, in clinical trials of HCV patients who were not responsive to IFN-α2a or IFN-α2b, there was a 30–50 % response rate.

## 7.8 Interferon-Gamma

Cells of the immune system produce interferon-gamma: dendritic cells, natural killer cells (NK), both CD4 and CD8 T-lymphocytes, and macrophages. Interferon-gamma is also induced by cytokines such as IL-1, IL-2, and by many growth factors. It is also induced during viral infections in vivo as a part of the immune system. IFN-gamma stimulates the expression of class I and class II MHC molecules and promotes the differentiation of naïve helper T-cells into Th1 cells, activates dendritic and cytotoxic T-cells, and increases the cytotoxicity of NK cells. Thus it is a key player in the immune response. (For an excellent short summary of IFN-gamma activities, see http://www.bio.davidson.edu/courses/immunology/students/spring2006/v_alvarez/ifn-gamma.html). IFN-gamma is an important component of cellular immunity and plays a significant role in cell differentiation, cell growth, and cell survival. In fact, if IFN-gamma were to be re-discovered today, it would not be called an "interferon" but rather an "interleukin."

IFN-gamma binds to specific receptors on the cell membrane known as IFN-$\gamma$R. The bound receptor interacts with enzymes, JAK kinase (Just Another Kinase), which phosphorylates STAT proteins, which in turn interact with specific DNA sequences upstream of inducible genes that have sequences in their upstream DNA, known as "GAS sequences" (gamma activated sequences—see Fig. 7.4). The spectrum of genes induced by IFN-gamma overlap, but with some differences from the genes induced by interferon alpha and beta [19]. One of the most studied genes induced leads to the production of a protein, IDO (indoleamine 2,3-dioxygenase). This enzyme catalyzes the rate-limiting step in tryptophan (an essential

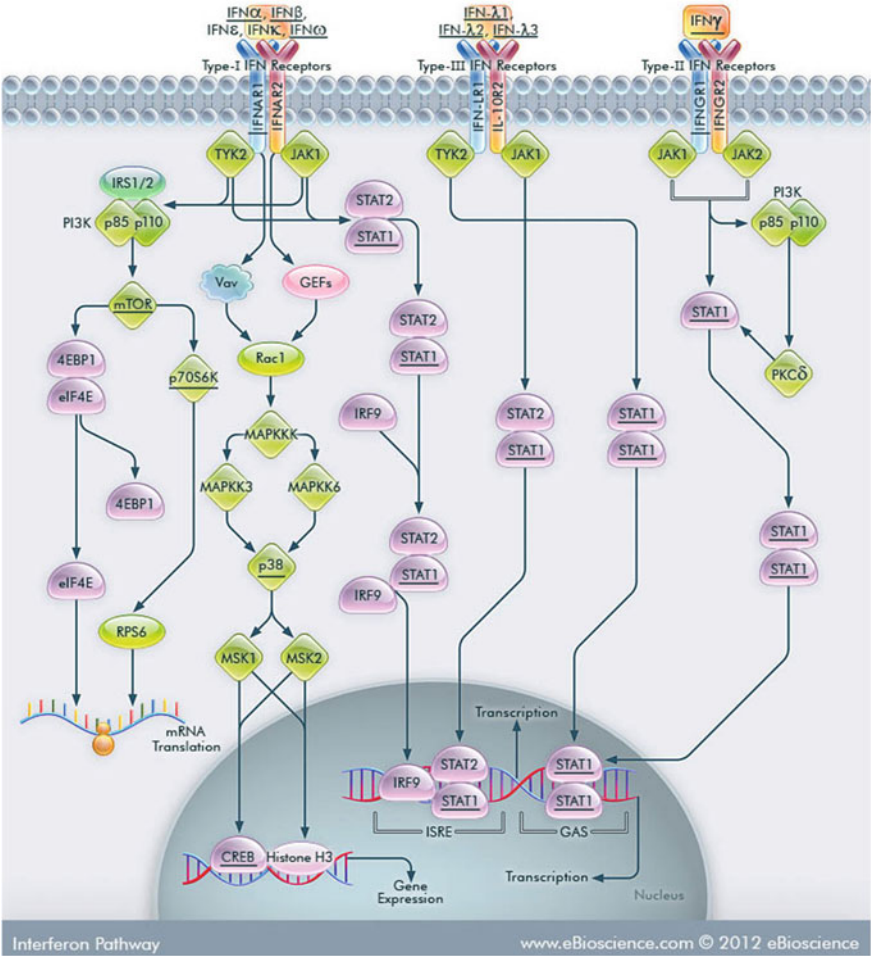


**Fig. 7.4** Simplified schematic of how IFN-alpha//beta, IFN-gamma and IFN-$\lambda$ stimulate gene induction (Courtesy of eBioscience, an Affymetrix company)

amino acid in humans) catabolism. In culture, cells die in the presence of interferon gamma, unless supplemented with tryptophan [20, 21]. It has been suggested that this enzyme is responsible for T-cell tolerance [22], is induced during pregnancy, and is important in the maintenance of pregnancy in humans [23]. However, this latter hypothesis is controversial [24, 25]. IDO may also have detrimental effects, being higher during septic shock and in certain types of cancers. Thus, the function of this enzyme, and many other proteins induced by the interferons, are still unknown.

## 7.9 Interferon Lambda: Type III Interferons

More recently, another group of interferons, called "IFN-lambda," has been discovered. Recent studies with IFN-lambda indicate that it is close in structure to the family of interleukins, IL10 and IL22 [26] and shares a common receptor (Fig. 7.4). It does have anti-viral activity, and can possibly replace IFN-α in the treatment of hepatitis C [27]. Since its discovery, the literature describing IFN-lambda has "taken off," and there are now hundreds of papers describing its biological activities, which overlap with the other interferons although binding to a different receptor. It may be useful in the treatment of viruses or cancers that have proven resistant to other interferons.

## 7.10 Interferon's Biological Activity

The complexity of the interferon system was reaffirmed when DNA microarrays were introduced (DNA micro-arrays measure mRNA changes following treatment of cells or whole animals or humans, contrasting two or more situations, such as treatment with a pharmaceutical agent against a control, or a cancer cell against a normal cell—see Fig. 7.5). Following treatment of cells with interferon-alpha or interferon-gamma, hundreds of genes are induced, yielding overlapping spectra for each type of interferon [28–30]. Many genes are also suppressed or "down-regulated" and most of the changes in gene expression are transient in vivo in humans. Many of these genes are well recognized as markers of interferon activity, but their function in regulating the response to virus is difficult to discern. It is becoming obvious that interferons are in fact part of a family of cytokines that are produced by one cell, diffuse to and act on nearby cells, and so drive the machinery of the immune response. Interferons induce many cytokines, which in turn induce many other genes producing a complex cascade of interactions. Cytokine biology is an area of biochemistry too complex to discuss here, but it must be emphasized that it has become an important component of immunology.

There are many questions that need to be answered in studying interferon at the cellular level. What happens after interferon binds to the cell receptor and triggers an anti-viral response? How does this response inhibit virus production? Has the virus developed methods of overcoming the interferon response? Are there specificity and differences among the various types of interferon? In answering these questions, one delves into the basis of the innate immune response and the relationship between the innate immune response and the induced cellular immunity. Answers to some of these questions are found in Chap. 6 (on immunology), and in Figs. 7.4 and 7.6.

As stated, interferons bind to specific receptors on the cell membrane. IFN alphas and betas both bind to the same receptors, IFNAR1, IFNAR2, and IFN-gamma bind to separate receptors, IFGNR1 and IFNGR2, and IFN-lambda to a

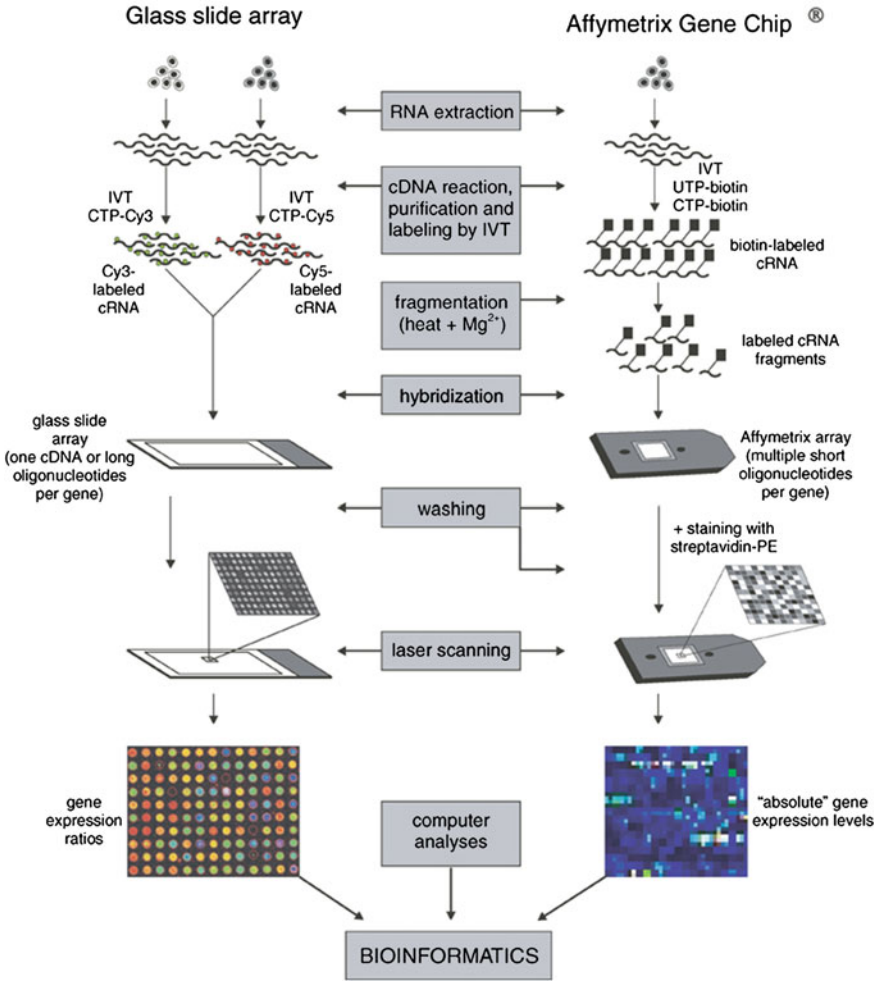


**Fig. 7.5** Hybridization to detect differential gene expression by DNA microarrays (Affymetrix)

third set of receptors which are shared with interleukins IL10, IL28A, IL28B, and IL29 [31] (see Fig. 7.4). Interestingly, virus infection also induces these interleukins. Binding of the interferons activates a number of kinases, including a series of proteins termed JAK kinases (Janus Kinase) which phosphorylate tyrosine on a group of proteins known as STATS (Signal Transducer and Activator of Transcription). The phosphorylation activates and leads to dimerization of these STAT proteins. There are seven STAT proteins that have been recognized, each related to a different function in either the immune response or metabolic response. The function of these STAT proteins has been elucidated from "knock-out" mice, i.e., mice with specific genes (in this case STATs), deleted. STAT1 is involved with the IFN-$\alpha/\beta$ and IFN-$\gamma$ signaling pathway, STAT 2 also with IFN-$\alpha/\beta$ pathway, STATs

3, 4, 5A and 5B, and 6 with other interleukins (cytokines). These STAT proteins in turn form complexes with other proteins that bind to specific DNA sequences. In type I interferon signaling, STAT1–STAT2 heterodimers combine with IRF9 (Interferon Response Factor 9, another family of transcription factors) to form ISGF3 (Interferon Stimulated Gene Factor), which in turn binds to the ISRE (Interferon Stimulated Response Element) promoter to induce what are termed interferon-stimulated genes. STAT1 dimers bind to another sequence known as a GAS sequence and interact with IRF1 to stimulate type II interferon genes (this is illustrated in Fig. 7.4).

In uninfected cells there is no trace of interferon; the gene is completely repressed. Virus infects a cell by binding to its unique receptor. After being taken up by endosomes or entering the cell through the cell membrane, viral components or viral dsRNA, which can only appear after initial viral replication, activate toll-like receptors or the analogous RIGI system. This is done through "pathogen associated recognition patterns," which interact with toll-like receptors. This interaction leads to kinase production, enzymes that phosphorylate proteins in a complex reaction that in turn activate genes for interferon production through complexes at specific sites on the promoters of the interferon genes, by a family of molecules known as interferon response factors (IRF-1, IRF-3, IRF-7). A similar family of proteins stops the induction and silences the interferon genes (Fig. 7.6). These proteins may function sequentially. Interferon can modulate immune responses by its effects on Class I and Class II MHC molecules (see Chap. 6).

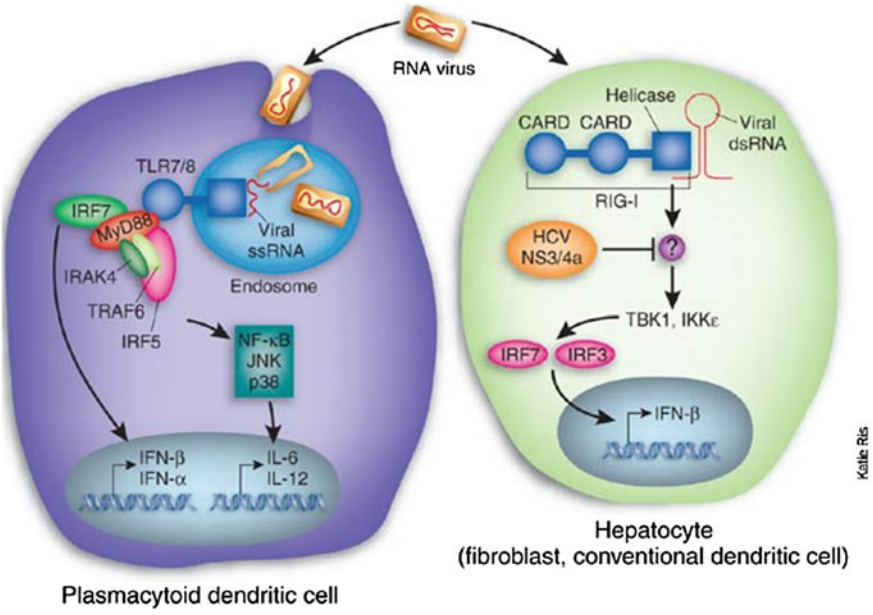


**Fig. 7.6** Toll-like receptor—and RIG-I-dependent—induction of type I interferon during RNA virus infection. Adapted from *Nature Medicine* **11**, 929–930 (2005) [32]

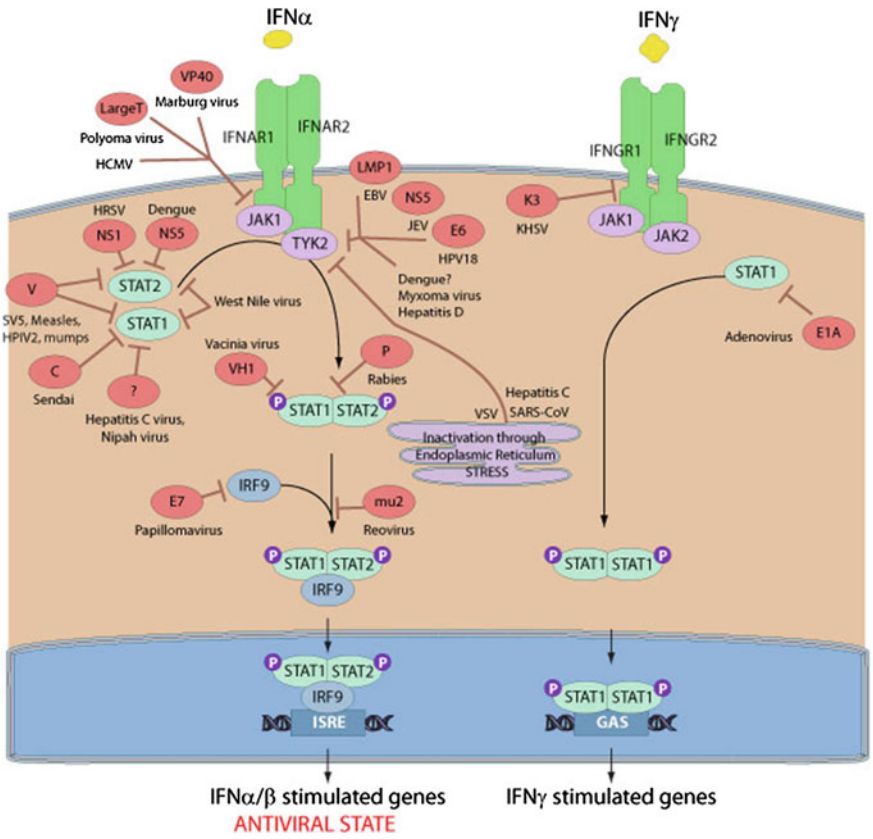


**Fig. 7.7** Antiviral pathway and steps at which viruses block IFN activity (Source: ViralZone www.expasy.org/viralzone, Swiss Institute of Bioinformatics)

IFN-alpha, IFN-beta and IFN-gamma increase expression of Class I molecules on all cells, thereby promoting recognition by cytotoxic T-cells that can destroy virus-infected cells. IFN-gamma can also increase expression of Class II MHC molecules on antigen-presenting cells, resulting in better presentation of viral antigens to $CD4^+$ T helper cells. Furthermore, IFN-gamma can activate NK cells and dendritic cells, which can kill virus-infected cells. As stated above, interferon induces many genes that are involved in gene regulation, cell differentiation, other cytokine induction and anti-viral activity. As discussed below, many viruses block interferon activity, as shown in Fig. 7.7.

Figure 7.6 illustrates the pathway of an RNA virus. Viral double-stranded RNA, an intermediate of virus replication, binds to proteins known as toll-like receptors, which are proteins that alert the cell to the presence of a pathogen. A complex forms on the toll-like receptor or RIG I, another toll-like receptor molecule, and interacts with a number of proteins that migrate to the nucleus and activate the genes for IFN, which in turn then activate nearby cells.

## 7.11 How Do Viruses Escape Interferon Activity?

Almost all viruses appear to have developed methods of evading interferon activity. These include inhibition of interferon biosynthesis, blocking interferon signaling, inhibiting the function of induced anti-viral proteins, and production of decoys to molecules that induce interferon signaling (Fig. 7.7). At the biochemical level, these include STAT protein degradation, inhibition of phosphorylation of STAT proteins, and IRF-3 inhibition. Both hepatitis B virus and HIV have been reported to block IFN-synthesis and signaling. The mechanism involved for all viruses in cell culture are described in [33]. During hepatitis C infection, there is robust interferon-stimulated gene transcription, [30, 34, 35] yet HCV persists to replicate to high numbers in chronically infected patients, unless treated with very large amounts of interferon and ribavirin. Under these conditions, some 70 % of patients will still produce virus. One report indicates that HCV does activate the enzyme protein kinase R (PKR), which normally would inhibit host protein synthesis by phosphorylating an initiating factor eIF2a inhibiting host protein synthesis, it appears to inhibit interferon stimulated gene production, thus dampening the interferon effect [36]. Using a cell culture system and an artificial viral construct (replicon, rather than complete virus), interferon activity was inhibited by viral proteins NS5A, E2 and by the IRES element of HCV [37]. This emphasizes the need to work with systems as close as possible to the "natural" infectious process, since in man none of these mechanisms may be functioning. Viruses such as hepatitis B, polio, SARS, adenovirus, and even HIV have been reported to inhibit interferon activity in cell culture, yet many of them are sensitive to interferon in man.

## 7.12 Clinical Studies of Interferons with Virus

Since 2002 interferon has been PEGylated to increase stability, with the addition of a polyethylene glycol molecule. The major use of interferon in the clinic has been in the treatment of hepatitis B and hepatitis C. In hepatitis B patients, the response rate is between 15–40 % of treated patients responding with long-term remission. IFN-alpha is used with a reverse transcriptase inhibitor, Lamivudin.

Until recently, the standard treatment for hepatitis C in patients was a 48-week regimen of interferon-alpha and the anti-viral drug ribavirin. The percentage of patients with no detectable virus after completion of treatment was approximately 30 %, depending on the type of interferon used, the antigenic type of the virus, and the race of the patients [38]. This protocol has recently been modified and usually includes a viral protease inhibitor.

Interferon has also been used in many clinical trials with inconclusive results. A small group of asymptomatic HIV-infected individuals were treated with IFN-α2b: 41 % had decreased viral titer, and no patients in the IFN-α group developed AIDS-defining opportunistic infection, compared with 5 patients in the placebo group ($P = 0.02$) [39]. However, 35 % of the patients in the treatment group

withdrew from the study because of the severity of the side effects. Other clinical trials have not been so successful. Attempts to treat children with Japanese encephalitis with IFN-alpha were unsuccessful, even though this virus and other flaviviruses are sensitive to interferon in cell culture [40]. During the SARS epidemic of 2002–2003, interferon was used among many other treatments, but the data are inconclusive [41]. Overall, the effect of interferon on virus infections in the clinical setting has been disappointing. Although interferon is active in inhibiting many viruses in cell culture, it does not carry over to the clinic, and there are many reasons for this. Most viruses induce interferon at an early stage of infection, so that the effect of interferon on the virus is established during the course of the infection, and additional amounts may not make much of a difference.

Even in the case of chronic infections, such as hepatitis C, interferon is induced and present during the infection [34]. It requires extremely high doses to have an effect on the virus, and at these levels IFN is quite toxic. In most clinical trials, a percentage of the patients will drop out of the trial. The experience with Japanese encephalitis and HIV indicate that once a viral infection has been established, it is difficult to eradicate the virus with interferon. Even in cells in culture, the timing of the addition of the interferon is crucial in inhibiting the virus. Long-term exposure to interferon—as in the clinical treatment of hepatitis C—may cause a decrease in white blood cells (leukopenia), leaving the patient susceptible to infections. Apart from the problems with the treatment, interferon therapy is expensive, i.e., $10,000–$20,000 for a 48-week regimen of interferon plus ribavirin.

## 7.13 Interferon as an Anti-cancer Agent

Although originally characterized as an anti-viral agent, to everyone's surprise interferon was also an anti-cancer agent. The first experiments performed were on mouse leukemia known to be of viral origin, including Friend's leukemia (identified by Charlotte Friend in 1957) and Rauscher's leukemia; both tumors later identified of retrovirus origin. However, interferon was not only active against tumors caused by viruses but against a large number of transplantable mouse tumors of different origins. In these experiments, tumors were injected intraperitoneally or intramuscularly, so that the interferon could be administered directly into the tumor.

Interferon later became a standard treatment for a number of types of human cancers, including hairy cell leukemia, Kaposi's sarcoma in AIDS patients, chronic myelogenous leukemia (CML), and papilloma infections (warts) [42].

By 1982 a phase 1 trial was conducted in a large group of patients with various cancers. There was a variety of side effects now known to occur with interferon therapy, including nausea, fatigue, headache, muscle pain, and occasionally elevated liver enzymes. However, there was objective evidence of antitumor activity in non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CML), Hodgkin's disease, breast cancer, and melanoma [43]. One of the first clinical uses of

interferon (IFN) was in the treatment of a rare leukemia known as hairy cell leukemia, which results from over-production of mature (and abnormal) B-cells. The cells under the microscope have a hairy morphology, hence the name. These cells in culture are very sensitive to interferon [44]. There are approximately 500 new cases of hairy cell leukemia each year in the U.S. Interferon (alpha, or beta, which was discovered later) is the therapeutic of choice, and about 80–90 % of patients will respond to treatment with remission for a period. Interferon therapy did extend the life span in early-stage melanoma and early-stage CML; however, in clinical trials it is inefficient against solid tumors. In Kaposi's sarcoma, as occurs in AIDS patients, it was effective if injected directly into the lesion, but not systemically. A major problem is the large dose of interferon required in any treatment as well as the severe side effects.

IFN-beta has been approved for the treatment of multiple sclerosis. The response to the drug varies and in some cases is ineffective, depending on the source of the interferon and the type of multiple sclerosis. Several studies have found IFN-$\beta$ beneficial in reducing rates of relapse, whereas others have reported no benefit in this regard [45].

## 7.14 The Interferon Society

The first meeting of those interested in interferon was held in 1964 in Bratislava (at that time in Czechoslovakia but today in Slovakia). There were sporadic meetings during the 1960s and early 1970s, but without an official organization. A report in 1974 claiming that interferon produced by Cantell could be used to treat cancer motivated a meeting in New York, organized by Mathilde Krim to stimulate interest in interferon and its anti-cancer activities. Dr. Krim was an activist in medical research, and was a faculty member of the Sloan Kettering Institute for Cancer Research. She was a well-known "socialite"in New York and her party for President Kennedy's 45th birthday was quite famous. She later became an AIDS activist, recognizing early on the problems and ethical dilemmas of the disease. (http://en.wikipedia.org/wiki/Mathilde_Krim). Bill Stewart, one of the early pioneers of interferon research, founded the Interferon Society in 1982. The first formal meeting and the publication of the *J. Interferon Research* occurred in 1983. The society met once a year with the presentation of papers on the three major classes of interferons ($\alpha$, $\beta$, and $\gamma$). The *Journal of Interferon Research*, only for papers on interferon, was established (although papers on interferon were published in other journals related to virology).

By 1989 the importance of the cytokines and their relationship to interferon was beginning to be realized, and the journal changed its name to the *Journal of Interferon and Cytokine Research* to encompass papers dealing with "other interferons," which were not called interferons but a variety of names based on their activities, such as the "tumor necrosis factor" (which was initially called lymphotoxin), IL-1, IL-2, etc. A separate Society of International Cytokine Research (ICS) was established in 1989. There is also a *Journal of Cytokine*

*Research*, which publishes papers on interferon research. We do not know how many cytokines (initially lymphokines, now interleukins) exist. The International Society for Interferon Research joined with the Cytokine Society in 2012 to form one organization for scientists working in both areas, which were obviously now interconnected. This would be known as the International Cytokine and Interferon Society. Many of the other cytokines (interleukins) are being tested for their effects on diseases and cancer.

## 7.15 Conclusions

The days of interferon as a clinical entity may be over. It has been replaced in the treatment of viral infections by small molecules that inhibit specifically viral enzymes, and such molecules may have fewer side effects. Interferon is still an important molecule to study since it elucidates the workings of the immune system. It is an important "backup" in the event of a sudden outbreak of an unknown virus epidemic; this was demonstrated during the SARS epidemic, when there was no alternative but interferon. There is not yet sufficient knowledge of the new interferons recently discovered, or whether they will have clinical applications.

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