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Interferons

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"Pooh!" cried Piglet "do you think it is another Woozle?" "No" said Pooh "because it makes different marks, it is either two Woozles and one, as it might be, Wizzle, or two, as it might be, Wizzles and one, if so it is, Woozle . . ."[1] The above quotation exemplifies the confusion experienced by many when first confronted with the interferons. The following is an attempt to simplify and summarise current knowledge of the subject.

It has been known for many years that infection with one virus may prevent infection of an animal or tissue culture with another, quite unrelated, virus—the classic example is that cells of the chorio-allantoic membrane which have been exposed to killed influenza virus are resistant to subsequent infection with live virus. In 1957 Isaacs and Lindemann showed that cells which were protected in this way produced a substance which was a humoral factor possibly responsible for the phenomenon. It protected other cells with which it was placed in contact and could, presumably, also protect the cells of the membrane that produced it. They called this substance interferon.

At the time of its discovery interferon appeared to be the perfect antiviral in as much as it was a natural product and was unlikely to be toxic; indeed, it was well tolerated by cultured cells and animals. Furthermore, it was active against a wide range of viruses. It is not surprising, therefore, that all the early work with this substance was concentrated on the study of its antiviral properties. Indeed, the activity of the International Standards for interferons were determined by their ability to inhibit virus multiplication in tissue culture and all putative preparations are assayed by comparison with such a standard. It is now realised that interferons possess many other functions and any one of a number of these—for example, inhibition of cell growth, modulation of the immune system may prove, eventually, to be of as great, or even greater importance, than their ability to inhibit virus replication.

Initial progress in the study of these substances was slow because of the limited amount of interferons that could be produced. Interferons show a marked species specificity for, generally speaking, they are only active in cells of the same species as that in which they are produced. In the last few years recombinant DNA technology has made possible the production of human interferons by bacteria and yeasts and the resulting increase in supplies has led to many more clinical trials being undertaken and the results of these are expected to be available shortly.

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What are interferons?

The Committee on Interferon Nomenclature (1980) decreed that "To qualify as an interferon a factor must be a protein which exerts virus non-specific antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein." The desired abbreviation for interferon should be IFN and qualified by the species of origin—for example, HuIFN for human interferon. Three types of human interferon are recognised at present, α, β, and γ, all of which are proteins and each is antigenically distinct. Naturally occurring interferons β and γ, but not α, are glycosylated.

HuIFNα, previously known as type 1 leucocyte interferon, and HuIFNβ, type 1 fibroblast interferon, are both stable at pH 2 while HuIFNγ, hitherto referred to as type 2 or immune interferon, is acid labile. All have monomer molecular weights of approximately 20 000; earlier reports of higher molecular weights for HuIFNγ have now been shown to have resulted from the presence of dimers. HuIFNα and β consist of 165–166 amino acids while HuIFNγ has 146. In each case most of the molecule, in its normal configuration, is required for its anti-

viral activity. Structurally, interferons α and β are similar and differ from interferon γ. Likewise, interferons α and β share a common cell receptor but that for interferon γ is distinct. Gene cloning has demonstrated a number of subtypes of interferon α, at least 12, but to date only a single, or possibly two, types of interferon β have been described although five mRNAs for its production are known to exist. HuIFNγ is restricted to a single type. The genes for interferon α and β contain no introns but that for interferon γ has three. Interferons are among the most active of biological substances with all three having specific activity in excess of 10^8 units per mg of protein.

How are interferons produced?

A wide variety of substances can induce the production of interferon, the most obvious of which is the animal viruses. It is immaterial whether the virus contains DNA or RNA or if it is a live or killed virus. They do, however, vary in their ability to stimulate interferon production with the paramyxoviruses (eg Newcastle disease virus, Sendai virus) and the arboviruses (eg Semliki Forest virus) being among the most efficient. Bacteria, phages, chlamydiae, rickettsiae, mycoplasmas, protozoa and fungi are among the other, mainly intracellular organisms, that can stimulate interferon production. Furthermore, the whole organism is not necessary for the induction of interferon production; fungal extracts, endotoxins, exotoxins and various lipid and sugar fractions such as capsular polysaccharide are also effective.

There are also a number of synthetic interferon inducers. The most notable of these is the ribopolynucleoside known as poly I:C. This compound and various chemical modifications of it suffer from two disadvantages, namely toxicity and sensitivity to nucleases present in animals and man. A number of anionic polymers and low molecular weight substances—for example, tilorone and acridine dye also stimulate interferon production in animals. In addition to their toxicity the use of all interferon inducers is restricted by hyporesponsiveness in that, with repeated use progressively less and less interferon is induced. Interferon γ is induced by the antigenic or mitogenic stimulation of T lymphocytes but the presence of macrophages is necessary for optimal production.

The genes controlling the production of interferon α and probably that of interferon β are situated on chromosome 9, and that for interferon γ on chromosome 12. The binding, uptake and processing of the inducer is followed by derepression of the interferon gene and transcription of interferon mRNA which is subsequently translated with eventual liberation of interferon. If the cell survives the action of the inducer interferon production rapidly declines, as the repressor re-exerts its influence. The time interval between the application of the inducer and the production of interferon varies. It may be as short as two to eight hours with early inducers such as poly I:C and bacteria or their products, or longer, six to 16 hours, with late inducers which include the viruses, tilorone and some anionic inducers. The production of interferon γ by T cells in response to antigens or mitogens takes very much longer, from 3–7 days, again depending on the inducer.

The yield of interferon from fibroblasts can be increased by one or two orders of magnitude by the process of superinduction. This consists of replacing the inducer (eg poly I:C) before the production of interferon by a reversible inhibitor of protein synthesis (eg cycloheximide) which allows the build up of interferon mRNA. The inhibitor of protein synthesis is then substituted by an inhibitor of RNA synthesis (eg actinomycin D) which prevents the formation of the mRNA of the protein repressor of interferon mRNA. This allows prolonged translation of the accumulated mRNA with increased production of interferon. The yield of interferon can also be increased by priming—that is, inducing cells which have already been exposed to small amounts of interferon. The greater quantity of interferon obtained under these conditions is related to the earlier production and more efficient translation of mRNA rather than its longer survival. Pretreatment with higher concentrations of interferon or repeated induction results in hyporesponsiveness which can be overcome, in animals, by the simultaneous administration of inducer and prostaglandins.

The classic methods for producing interferons for clinical trials have been as follows:

1 HuIFNα was produced by exposing the buffy coat of human blood to Sendai virus—hence its name leucocyte interferon.
2 HuIFNβ fibroblast interferon resulted from the induction of human fibroblasts by poly I:C and the yields increased by superinduction.
3 A mixture of mainly HuIFNα but some HuIFNβ was produced by lymphoblastoid cells following induction with Sendai virus.
4 HuIFNγ was produced by T lymphocytes in response to stimulation with mitogens.

The amount of interferon α and γ that can be produced by these methods is limited by the availability of leucocytes. HuIFNβ production is restricted by the surface area of glass required to support the growth of fibroblasts. Lymphoblastoid cells will grow in suspended cultures and, if a high interferon yielding line such as Namalwa is selected, far greater

amounts of interferon can be produced than from attached-cell-cultures. However, recombinant DNA technology has proved the most satisfactory route to the large scale production of human interferons. The genes of all three types of HuIFN have been cloned in micro-organisms and expression obtained. HuIFNβ and γ produced in this manner lack the glycosylation present in the naturally occurring substances but this does not affect their specific activity. Greatly improved methods of purification, including immunoadsorption chromatography on monoclonal antibody columns, are now available so there should be no difficulty in supplying adequate amounts of very pure, high titred interferon of all three types although, up till now, only HuIFNα has been readily available.

What do interferons do?

Originally it appeared that interferons were simply antiviral substances produced in response to virus infections but, just as it is now clear there are many other inducers of interferon, so it has been discovered that interferons possess many additional activities. The two properties of interferon which have attracted most attention are those with the greatest clinical potential, namely, their antiviral activity and their ability to inhibit cellular growth and division.

Interferons do not act directly on viruses but protect cells by inducing an antiviral state which takes time to develop and is dependent on both RNA and protein synthesis. This antiviral state is wide-ranging, inhibiting virtually all viruses and many other organisms which, like those capable of inducing interferon, are mainly intracellular parasites. This protective effect is, on the whole, species specific although there are examples of cross-species activity where interferon produced in the cells of one species has an effect on the cells of a heterologous species eg HuIFNα on mouse cells and vice versa. The antiviral state inhibits virus multiplication at various stages depending on the virus involved and, while the major inhibition may be at the level of transcription of viral RNA, translation of mRNA or viral protein synthesis, interferon also affects the maturation and release of enveloped viruses and may even influence the mechanism by which the infecting virus is uncoated. Two other properties of interferon which influence the production of interferon and, therefore, the antiviral state of cells—that is, priming and hyporesponsiveness, have been described already.

Both the original partially purified and, more recently, highly purified preparations of interferons α, β and γ have been shown to inhibit the division of both normal and transformed cells in vitro and in vivo. However, different cells vary in their responsiveness and the rate at which they recover once interferon is removed. There is no toxic or lytic element to the antiproliferative effect of interferon. Interferon given daily to newborn mice results in a failure to gain weight and a similar inhibition of weight gain was seen in children with congenital cytomegalovirus infection when they were treated with interferon. In mice it has been shown that, not only will interferon inhibit the development of Friend and Rauscher leukaemias caused by viruses, but also the growth of transplantable tumours of non-viral origin. This suggests that the effect of interferon on tumours is not entirely caused by its antiviral properties but that it possesses also the ability to inhibit the growth and division of cells. Interferon has a protective effect on mice inoculated with interferon-resistant mouse leukaemia cells although this is less than that in mice injected with interferon-sensitive leukaemia cells. However, that mice inoculated with interferon resistant cells and treated with interferon survive longer than control mice indicates that interferon's protective action is mediated by the host response in this instance. A knowledge of these facts is all important when considering the potential value of interferon in treating human tumours for very few are known to have a viral aetiology. In animal experiments the antiproliferative effect is dose related, the more interferon given the greater the effect, and the best results have been obtained with frequent administration over lengthy periods.

Recently, increasing attention has been paid to the immunomodulatory powers of interferon for it is recognised that, in addition to any manifestation that may be directly attributable to this function of interferon, changes in the behaviour of the immune mechanism could account, in part, for both the antiviral and antiproliferative effects of interferon. Interferon influences both humoral and cell mediated immunity. It has been observed in mice that interferon present before the antigenic stimulus is received results in inhibition of the antibody response irrespective of whether that response is a primary or a secondary one, whereas, interferon given after antigenic stimulation enhances the antibody response and also the B cell population. However, somewhat different results have been obtained by others and in human cells the reverse appears to apply. Perhaps the most that can be said at the present time is that interferon influences the antibody response but the precise way in which it does so depends both on the amount given and the time it is given in relation to exposure to antigen.

T cells are also affected by interferon as demonstrated by the enhanced lytic action by sensitised T

cells, killer cells, in its presence. A similar effect has been observed on the non-B, non-T, lymphocytes known as natural killer cells, which are cytolytic in the absence of antibody. On the other hand interferon renders normal cells more resistant to natural killer cell activity and this may account for the selective activity of natural killer cells on tumour as opposed to normal cells.

The phagocytic action of macrophages is also increased by interferon treatment of the host, for both inert carbon particles and tumour cells are more speedily removed from the peritoneal cavity of animals in the presence of interferon. However, interferon has been shown to inhibit the maturation of human monocytes to macrophages and an increased sensitivity to this inhibiting effect in subjects with trisomy 21 has led to the suggestion that it may be responsible for such conditions as the increased carrier rate of hepatitis B in those with Down's syndrome.

It has long been accepted that viral infections, such as measles, can be associated with a diminished Mantoux reaction. A similar suppression of the delayed hypersensitivity reaction results from the administration of interferon, especially if it is given before the antigen. Interferon's effect on graft rejection, as on the antibody response, is variable and depends on both the amount used and the time it is given in relation to the time of grafting. A small dose of interferon, given before grafting, may accelerate rejection while a larger dose will delay rejection. Administration of interferon after grafting can result in the early rejection of the graft because of inhibition of suppressor T cell activity.

Interferon also affects cell membranes by increasing their density and producing changes in the protein to lipid ratio and the fatty acid composition of the phospholipid. The cell size and cytoskeleton are altered and there is a decrease in cell motility. There is an increase in the net negative charge of interferon treated cells. One biological result of these changes is observed in the inhibition of release of oncornavirus and vesicular stomatitis virus in much the same way as plasminogen fails to be released from interferon treated cells. Virus particles released from interferon treated cells may also be less infectious than those from untreated cells. Interferon enhances the expression of certain cell components notably the HLA-antigens and β_2-microglobulin.

Interferon can enhance the toxic effect for cells of certain substances particularly dsRNA of both natural and synthetic origin. Similarly, vaccinia virus causes early lysis of cells treated with interferon but not of untreated cells. The enhanced toxicity of concanavalin A for interferon treated cells is probably related to the increased amount of concanavalin A which is bound to such cells compared with normal cells.

Interferon also influences the synthesis of various cell products, some being depressed while others are enhanced and an induced product, interferon, may be enhanced (primed) or inhibited (hyporesponsiveness) according to the amount and frequency with which the inducer (interferon) is administered. Hyaluronic acid and prostaglandin E production along with that of tRNA methylase, protein kinase and 2′, 5′-oligoadenylate synthetase can be enhanced by interferon but a number of enzymes—for example, tryosine amino transferase, glutamine synthetase and ornithine decarboxylase are inhibited.

When signs of toxicity were first noted with partially purified interferon it was convenient to incriminate impurities in the preparation as the responsible factor. Pure interferon has now been given to sufficient subjects, with only a minimal reduction in the toxic effects, to be able to attribute these signs and symptoms to the interferon itself. Perhaps it is not surprising that they do occur for, in nature, interferon production is rigidly controlled so that cells are only exposed to its action for relatively short periods.

If more than 10^6 units of interferon are given intramuscularly then a febrile reaction occurs which consists of fever, sometimes rigors, headache, malaise and myalgia. It has been suggested that the constitutional symptoms which are such a prominent feature of influenza, a respiratory infection, are caused by the presence of endogenous interferon. The febrile reaction becomes less with continuous administration of interferon which induces anorexia and fatigue and it is this latter symptom which restricts the amount of interferon that most individuals can tolerate. Interferon inhibits myeloid progenitor cells and, larger doses, the erythroid progenitor cells so that leucopenia is common in interferon therapy although it is readily reversible on cessation of treatment. Anaemia and thrombocytopenia are less common. Hepatic transaminases may also be reversibly raised. Less common toxic manifestations include loss of hair, diarrhoea and nausea or vomiting, and central nervous system symptoms of parasthesia, drowsiness and even coma with very high dosage schedule. Local erythema follows intradermal inoculation of interferon, stuffiness and increased secretion when given intranasally for prolonged periods and an increase in CSF protein and cells when administered intrathecally.

Mouse but not human interferon given daily from birth for the first week or so of life can produce stunting, liver necrosis and death in mice and

glomerulonephritis of the immune complex variety in survivors. Virtually the same syndrome occurs in newborn mice infected with lymphocytic choriomeningitis virus suggesting that the disease could be the result of exogenous interferon in the former and endogenous interferon in the latter. If antimouse interferon serum is given at the same time as virus to newborn mice the viraemia increases, the interferon concentration is reduced, the mice gain weight, liver necrosis does not occur and the onset of nephritis is delayed. Furthermore, the virulence of lymphocytic choriomeningitis virus for different strains of mice has been shown to be related to the amount of interferon that this virus induces in that strain. There is, thus, strong evidence that interferon can produce disease, at least in mice.

How do interferons work?

It was said earlier that the antiviral and antiproliferative activities of interferon are considered the most important and have received the most attention. With some knowledge of the capabilities of interferon it is worth while looking to see what progress has been made towards understanding the mechanism whereby interferon achieves its results in these two spheres.

The induction of the antiviral state of cells requires binding of interferon to the cell receptor and probably as little as one molecule a cell can be effective. Whether interferon needs to enter the cell is unproven but time, the cell nucleus and RNA and protein synthesis are all essential. At which stage during virus infection inhibition occurs is more difficult to determine; not only does the interdependence between viral RNA and protein synthesis present problems but the results obtained have been confusing, even contradictory, when different viruses have been employed. This has led to the general acceptance of a multisite theory for the action of interferon so allowing interferon to render cells resistant to one virus but susceptible to another depending on which mechanisms are stimulated by interferon in that particular cell and whether those mechanisms are important in the replication of the virus studied.

In interferon treated cells the effect on uncoating of the virus appears to be minimal, certainly with positive-stranded RNA viruses, although SV40 may be inhibited at this stage. What happens at the stage of virus transcription is much more complex but may be summarised as, in RNA virus infections, especially those with their own polymerases, there is inhibition of early transcription of viral RNA but this is insufficient to account for the whole of the antiviral activity. With DNA viruses there is no inhibition of transcription with vaccinia virus but, under certain conditions, it may be observed in SV40 virus infections.

Irrespective of whether there is inhibition of virus transcription or not there is inhibition of viral protein synthesis in virtually all virus infections of interferon treated cells and this has best been demonstrated with vaccinia virus. A number of possible ways in which interferon treated cells could inhibit protein synthesis are known and involve two dsRNA dependent enzymes, 2′, 5′ A synthetase and a protein kinase, 67K, inhibition of cap methylation and possibly, a deficiency of certain tRNAs; each of these will be considered in turn.

2′, 5′A synthesase production is enhanced by interferon treatment of cells, is activated by dsRNA and catalyses the production, from ATP, of oliogoadenylate with a 2′, 5′ linkage that activates a nuclease which hydrolyses mRNA so inhibiting protein formation.

Protein kinase, 67K is also induced by interferon treatment of the cell, activated by dsRNA and phosphorylates the α subunit of the protein synthesis initiation factor known as eIF2 which is thereby rendered inactive and protein synthesis is inhibited.

Inhibition of cap methylation makes mRNA more vulnerable to degradation and reduces its efficiency at binding to ribosomes so impairing its ability to act as a template for protein synthesis.

A deficiency of certain tRNAs has been noted in the lysates of interferon treated cells so that elongation of protein molecules is prevented and they are prematurely terminated.

Finally, the changes in the cell membrane of interferon treated cells inhibit the release and infectivity of the progeny of enveloped viruses. In some, mouse mammary tumour virus, the virus particles have normal morphology but remain attached to the host cell while in others, vesicular stomatitis virus, the particles lack the peplomers whereby they attach themselves to cells and are, thus, non-infectious. Yet other viruses, mouse leukaemia virus, are released normally and have normal morphology but are less infectious than virus from untreated cells, possibly as a result of a deficiency of glycoproteins in the virus from interferon treated cells.

These are all possible methods by which the inhibition of the production of new infectious virus progeny may occur in interferon treated cells but to what extent each is active in infections with different viruses remains to be determined. Similarly, there are numerous ways in which the antitumour effect of interferon can be explained. It has already been stated that interferon has a direct inhibiting effect on cell growth and division and that tumour cells are more vulnerable than normal cells. 2′, 5′ A synthetase has been detected in many mammalian cells and

the amount present varies with growth. It is possible, therefore, that interferon may exert its growth inhibiting activity through the 2′, 5′ A system limiting protein synthesis. A further effect of interferon on tumour cells is to increase the exposure of surface antigens. This, in conjunction with interferon's action on the host—that is, the increase in cytolytic cells, especially natural killer cells, and macrophages could, and probably does, contribute to the antitumour effect. The action of interferon on tumours represents the summation of its effects on the tumour and those on the host.

Clinical application of interferon

Interferon, known for over 25 years to possess dramatic antiviral properties in vitro and for a somewhat shorter period to have antiproliferative activities, has yet to find a recognised place in the routine prevention and treatment of human disease. This may appear surprising but it must be remembered that, until recently, adequate supplies of potent, pure interferon were not available. The results of early experiments were often disappointing because of the small amount of interferon that was given and, when success was reported, there were doubts as to whether this should be attributed to the interferon itself or to impurities present in the preparations. Furthermore, in many of the trials controls were inadequate or completely absent, understandably when treating cancer patients for there was a natural reluctance to withhold a potentially beneficial drug in a fatal disease, but, nevertheless, making the interpretation of results more difficult.

What could be expected from the use of exogenous interferon in human infections? It has been shown that, in uncomplicated, natural influenza infections, the kinetics of interferon production parallels that of virus and clinical symptoms and that antibody, including IgA, only appears as the illness abates, and rises during convalescence. It is also known that interferon does not affect the virus directly but acts by protecting the host cells. It would seem therefore, that the greatest benefit of interferon is most likely to be seen when it is given prophylactically. By the time symptoms are apparent in influenza, endogenous interferon production is approaching its zenith and little would be expected from giving additional exogenous interferon at this time. Should there be a deficiency of interferon production, as has been shown to occur in a proportion of young children who suffer repeated respiratory infections, then dramatic results can be anticipated from replacement therapy with exogenous interferon. Success with interferon could result from its use in chronic infections, or where the immune status of the individual is impaired, for in these instances there must be unprotected, susceptible cells available in order to maintain the infection.

Knowing which types of infection are likely to respond to interferon leads one to consider which type of interferon to give. In the past this has largely been determined by which interferon, α or β, was available (virtually no studies have been done with interferon γ). Although similar in many respects these two interferons differ in their pharmacokinetics. Following intramuscular injection, interferon α serum concentrations reach a peak after 4–12 h and can still be detected after 24 h. However, interferon β has to be given in very much larger doses to be detectable at all in the serum because it is bound locally, and possibly destroyed, in the muscle. Both interferon α and β, when given intravenously, result in immediate serum concentrations which rapidly fall over the following 8 h. While such considerations help to determine the route and frequency with which it is necessary to give interferon, the actual amount required has often been gauged by trial and error. Sufficient trials have now been completed with pure interferon to be reasonably certain that the results obtained with partially purified material were the response to the interferon they contained and not caused by impurities.

Interferon has now been tried in many of the viral infections which fall into the three categories outlined above, interferon deficiency, prophylaxis, and chronic infection or infection in those with impaired immunity, and the findings are summarised here. Patients with fulminating hepatitis who appear not to produce interferon, for it is not detectable in the blood, respond to exogenous interferon by producing their own; possibly an example of exogenous interferon priming the host cells.

Intranasal interferon, given two or three times a day, will protect volunteers against experimental infection with the more usual aetiological agents of the common cold, ie rhinoviruses and coronaviruses. Most colds are so trivial that it is only on rare occasions and for short periods that one would envisage the need to protect normal individuals against infection. However, for those in whom a cold could lead to a more serious illness—for example, asthmatics and chronic bronchitics, protracted protection throughout the winter months would be required and the preparations tested so far produce unwanted side effects in too high a proportion of subjects to make them acceptable for long-term prophylaxis. We must wait to see if either interferon β or γ has a greater therapeutic index which would enable it to be used for this purpose.

Interferon α and β have been used in an attempt to limit the spread of dendritic ulcers of the cornea

which result from herpes simplex virus infection and are often recurrent because of reactivation of the latent virus. By itself interferon is ineffectual but in combination with some form of debridement or a nucleoside analogue, such as trifluorothymidine, more rapid healing results than that occurring in the absence of interferon. Interferon has also been shown to be effective in reducing the incidence of herpes labialis which often follows the section of the trigeminal nerve for the relief of pain in tic doloureux. However, interferon had no influence on the rate of spontaneous reactivation of herpes simplex virus in these patients.

It would not be expected that exogenous interferon would make much impact on the lesions of either herpes simplex or varicella-zoster virus once they had reached the vesicular stage for such lesions contain a high concentration of interferon. However, in immunologically compromised patients the appearance of interferon in the vesicles is delayed. In patients with malignancies who develop shingles exogenous interferon can reduce the time to healing of existing lesions, dissemination of virus, serious complications and pain, both during the acute phase and by a reduced incidence of post-herpetic neuralgia. Similar results have been obtained in immunosuppressed children with varicella. Although interferon can reduce, or suppress, the excretion of cytomegalovirus in the urine of some congenitally infected children and delay the onset of cytomegalovirus viraemia in renal transplant patients there is no evidence that this materially alters the course of the illness. Congenital rubella is another chronic infection in which interferon has been tried but the results have been as disappointing as those obtained in congenital cytomegalovirus infection.

Hepatitis B is an infection in which a minority of cases progress to a chronic hepatitis, possibly as the result of a deficiency in the immune response, and such a state is, therefore, a candidate for interferon therapy. Parenteral interferon on a daily or thrice weekly basis can produce a transient fall in concentration, or elimination, of the markers of active viral replication, ie HBcAg, HBcAb, HBeAg, Dane particles and HBV DNA polymerase. Repeated courses of interferon can result in halting the disease process and eliminating all the markers in a proportion of cases and this proportion can be increased by combining interferon therapy with a nucleoside analogue. However, there are also many who fail to respond.

We now come to the crossroads of interferon's two potential clinical uses, ie tumours of viral origin. In this situation interferon may act in any of three ways: antiviral, antiproliferative or by modulating host cell mediated immune response. The commonest virus-associated tumour of man is the simple wart caused by a papilloma virus. Interferon injected into the lesion will cause regression but systemic interferon has little effect, probably because interferon given systemically produces a very low concentration in the lesion itself. Juvenile laryngeal papillomas are rare benign tumours of children which have required repeated surgical removal to maintain a clear airway. Although interferon has no dramatic effect on the tumour once established it will prevent recurrence after surgical removal but interferon α appears to be more effective than interferon β. However, unless sufficient interferon is given tumours recur as they do when medication is stopped and it still needs to be determined what proportion of subjects with juvenile papillomas will respond and for how long therapy must be continued to prevent recurrence. Genital warts and juvenile laryngeal papillomas contain few virus particles and show infiltration with mononuclear cells which is the reverse of that seen in non-genital warts. As the former are more responsive to interferon treatment than the latter it would suggest, in this instance, that the effect of interferon on the cell mediated immune response is the important distinguishing feature.

The antitumour effect of interferon that can be demonstrated in vitro and in animals has led to attempts to control human cancers by this means. The original, uncontrolled trial of interferon in osteogenic sarcoma following surgical removal showed an improved survival rate compared with the results obtained in previous years at the same institution. However, it became apparent that in the interval survival rates had improved at other places where interferon was not used. Nevertheless, these results together with a natural desire by all concerned to find an effective agent against a common and fatal condition, encouraged the study of the effect of interferon on other forms of cancer. Results available to date are mainly from trials conducted with natural, leucocyte interferon. Although there are instances of dramatic cures such as the recurrent nasopharyngeal carcinoma (an EB virus associated tumour) that failed to respond to all other treatment but rapidly resolved when given interferon, such examples are rare. The more common reaction, even in the most responsive conditions—for example, myeloma, non-Hodgkin's lymphoma and breast cancer, is partial regression of the tumour and then only in a proportion of cases. In other malignancies the proportion of cases showing any response is very low indeed. However, it must be recognised that the patients selected so far have been those with advanced disease and often resistant to other forms

of treatment and it would be expected, from animal experiments, that interferon is much more likely to be successful when the tumour load is small. Furthermore, the quantity of interferon given has been empirically determined, often selecting the maximum tolerated dose, but it could well be that smaller doses or differing intervals between administration would have a greater effect on the immunomodulatory power of interferon if this is the way it functions in a particular malignancy. It would be wise to await the results of the numerous trials currently in progress before attempting to pass a verdict on the role of interferon in cancer. However, the fact that interferon has some effect, in some cancers, in some individuals, gives hope that with a greater knowledge of its mode of action and given early, possibly in conjunction with other therapy, interferon may contribute more to the control of malignancy in the future than has been suggested by its performance in the past.

Interferons have proved to be more complicated substances than they first appeared to be but, so far, have failed to live up to the, probably over enthusiastic and optimistic, role envisaged for them in clinical medicine. There can be no doubt that interferon is not the panacea many believed it could be. However, successful prophylaxis of some viral infections, halting of the disease process in others and regression of certain tumours has been achieved by the use of interferons. We are only at the beginning of an understanding of these substances and, as with any new drug, there is the need to determine the optimal dosage and identify those conditions in which it will have a beneficial effect. To date work has, by necessity, been confined to relatively impure preparations or pure preparations of interferon α. The exact performance of pure interferon β, interferon α sub-types and interferon γ has yet to be determined and it is possible, certainly with interferon γ, that one or more, either alone or in combination, will prove a more effective therapeutic agent than any tested so far. An enormous amount of work has been performed with interferons which has provided much useful information on many basic reactions at the molecular level but without providing a full explanation of how interferons work. A better understanding in this area could allow a more rational approach to interferon therapy.

New viral diseases, or established ones which suddenly come to prominence because of the changing pattern of living, can constantly be expected. Acquired immune deficiency syndrome is one such disease and is a good candidate for interferon therapy, not only because of its probable viral aetiology and immunodepressed state, but also because the terminal stages are often associated with tumours, Kaposi's sarcoma. However, restraint must be exercised in the clinical application of interferon. Interferon has been detected in the serum of patients with certain autoimmune and allergic conditions and the administration of interferon to such patients could have disastrous consequences. These effects may not be seen for years, in much the same way as glomerulonephritis occurs late in mice infected with lymphocytic choriomeningitis virus at birth, and antibody to interferon, rather than interferon itself, may be more appropriate. In these diseases it is important that the cause and role of the circulating interferon be firmly established before therapy is undertaken.

Interferons are extremely active and powerful biological substances that could yet prove a most useful component of the clinician's armory but caution is needed when attempting to realise their full clinical potential.

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Notes