Animal models in interferon research: Some current trends

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Introduction

This is not a comprehensive review of all animal studies performed with interferon over the years. The reader is referred to a number of other papers published recently in which we covered different aspects of animal studies [37, 38]. I want to concentrate on the in vivo studies in which the mode of action has been studied and the in vivo studies in which the activity of interferon on microbiological infections other than virus infections has been tested.

The in vivo mode of action

Interferon was discovered because of its antiviral activity in vitro [17]. Interferon preparations are still quantitated on the basis of this activity. Since its discovery, however, many more activities have been described and the question now is how relevant these activities are for the activity of interferon in vivo [8]. For a number of reasons it is important to find this out.

Knowing the mode of action will make it easier to identify those diseases in which interferon treatment makes sense. It may also reveal which in vitro assays are relevant to the activity of interferon in vivo. Because of the development of recombinant DNA technology it is now possible to make an enormous number of so-called 'second generation interferons' by manipulating the genes for the different interferons. Suitable in vivo assays are essential to screen these interferons for their potential activity in man. Establishing the host mechanism activated by interferon is also important in order to monitor the efficacy of interferon treatment in individual patients and to devise more rational treatment schedules. At present there is more than a billion-fold difference in the amounts of interferon reported to be necessary to induce different biological effects in vivo [7, 11]. It is obvious that under such circumstances any rationale for the application of interferon in man is lacking.

The antitumor action of interferons in vivo

The discussion regarding the mechanism of the antitumor activity of interferon has focused on the question whether direct effects on the tumor cells or host-mediated mechanisms are responsible in vivo. Already soon after the discovery of interferon it was shown to inhibit cell multiplication in vitro [30]. Both the antiviral effect and the anticellular effect are the result of a direct interaction of interferon with its receptor on the cell membrane. It was assumed initially that the antitumor effect in vivo was the result of these direct effects. The efficacy of interferon in virus-induced tumors, the first animal models in which the anticancer activity of interferon was tested, seemed to confirm this view [23].

In 1972 Gresser and coworkers reported that interferon treatment increased survival in DBA/2 mice inoculated with *L1210* leukemia cells resistant to interferon in vitro [10]. This indicated that, in certain tumors at least, host-mediated mechanisms were responsible for the in vivo efficacy rather than direct interactions between interferon and the tumor cells.

Over the years Gresser's group has published a number of papers on their detailed studies in another model system in which they used Friend leukemia cells (FLC) [1, 2, 12, 13]. FLC are transformed erythroid stem cells. They employed a clone of FLC which was resistant and a clone which was sensitive to interferon. Ten [10] units/ml interferon were enough to induce an antiviral state or to inhibit cell division in the sensitive clone. The resistant clone did not even respond to 10^5 units/ml. Both clones induced tumors in DBA/2 mice and proved equally sensitive to interferon treatment in vivo [1]. Interferon treatment resulted in a decreased number of colony-forming tumor cells in the peritoneum and an increased survival. No change in phenotype occurred after in vivo passage. The interferon-resistant clone proved also to be resistant after re-isolation from the peritoneum. There was no evidence that interferon stimulated the differentiation of the FLC in erythroid cells.

Using radiolabelled cells, Gresser and his colleagues were able to show that cell destruction in the peritoneum caused the disappearance of the FLC [2]. They were not able to pinpoint the host mechanism responsible for the effect. It was impossible to transfer the antitumor activity from mouse to mouse by peritoneal washings. Injection of silica particles, which blocks the activity of phagocytic cells, had no effect on the in vivo activity of interferon in this system. Reducing the treatment to the period before inoculation with FLC proved less effective than treatment after inoculation. Therefore the host mechanism responsible for the effect obviously needed constant stimulation by interferon for maximal activity.

Interferon proved to be most active when inoculated at the same site as the tumor cells [12]. There was also a direct relation with the total amount of interferon given. The

more interferon, the better the effect. The spacing of the interferon treatment proved to be important. Larger amounts administered with longer intervals proved less effective than smaller amounts give more frequently. The number of tumor cells inoculated also influenced the efficacy of interferon. The smaller the number of cells inoculated, the greater the effect of interferon.

Recently, Gresser et al. reported on the effect of interferon on metastases in this system [13]. When DBA/2 mice were inoculated subcutaneously with FLC and the resulting primary skin tumor was removed when it became palpable, after 8–9 days, the animals died within 24 to 28 days by metastatic spread of tumors in the lungs and the spleen. Daily treatment with large amounts of murine interferon alpha/beta for 52 days resulted in an increase of survival to 55–65 days. Also in this model the interferon-sensitive and interferon-resistant FLC proved to be equally sensitive in vitro.

Another method of discerning the direct and indirect antitumor effects of interferon in vivo has been the use of human xenografts in nude mice. In this model, human tumor cells are injected, and they form a tumor which the mice cannot reject because of the lack of an intact immune system. If these animals are treated with human interferons the effect is the result of direct interaction between the interferon and the tumor cells. A number of elegant studies on this model have been studied, especially by the group of Frances Balkwill. We have reviewed her studies and others recently [37], so I will restrict myself to the main conclusions.

A number of human tumors are sensitive to human interferon in this system, indicating that direct effects can be important for the effect in vivo. In this system also, discrepancies were reported between the in vitro and in vivo activity. The pharmacological behavior of human interferons in mice may well explain these differences. We noted a striking difference between the homologous mouse system and the xenograft-nude mouse system. In the homologous system, in which transplantable mouse tumor cells are injected, the interferon (of course of mouse origin) is only active when the tumor load is low and the interferon is given early. In general, no growth inhibition is noticed in established tumors. In nude mice with human tumors, and treated with human interferon, tumor reduction has been reported. This could be a property of the human interferon system because tumor reduction by interferon can also be achieved in cancer patients with e.g. Kaposi sarcoma or hairy cell leukemia.

There is a large variation in the sensitivity of xenografts to interferon, and there is no relation with tumor type. The response of a tumor in the xenograft system cannot be extrapolated to indicate the clinical efficacy in patients.

So, in conclusion; the mode of action of interferon against tumors in vivo remains a mystery. And it cannot provide an answer to basic questions about what tumors to treat with which treatment regimen.

The antiviral activity of interferon in vivo

For the antiviral activity of interferon, also, there seems to be no relation between the activity in vitro and in vivo. In 1979 we showed that a vaccinia virus, which was not sensitive to human interferon in vitro, did not induce skin lesions in rhesus monkeys treated with human interferon [34]. This result indicated that host-mediated mechanisms also played a role in the antiviral efficacy in vivo. This is not true for viral infections and not even for gamma interferon. This interferon plays an important role in the immune system and is considered to be more a lymphokine than an interferon. Rat gamma interferon is capable of protecting rats against a lethal infection with pseudorabies virus. We have used pseudorabies-infected rats to study a number of problems relating to the in vivo antiviral activity of interferon [35, 36, 39, 40]. We assumed that gamma interferon exerted its action through the immune system of the host. The primary target of the virus after i.p. infection is the serosal cell of the peritoneum. From there it spreads via the neural plexuses of the gastrointestinal tract to the central nervous system.

In rats treated with gamma interferon no virus could be detected in the primary target cells. Primary cultures of serosal fibroblasts from the peritoneum of rats proved to be sensitive to the antiviral activity of gamma interferon, but peritoneal macrophages were not. We have studied the antiviral efficacy in vivo in pseudorabies-infected rats in which the host defence mechanisms were blocked by a number of different immunosuppressive or macrophage-blocking regimens. In these animals as well as in newborn rats in which the immune system is still immature, and in nude rats lacking a functioning immune system, the antiviral activity of rat gamma interferon was as efficient as in normal rats. So we assume that in this model, at least, gamma interferon exerts its antiviral activity by direct interaction with the target cell of the pseudorabies virus.

Also at the level of the different interferon subtypes, there is no correlation between the activity in vitro and in vivo, as we and others have shown [33, 37, 41]. A recombinant hybrid human alpha interferon which showed 100 times more antiviral activity in vitro than natural human alpha interferon was much less active than the latter in vaccinia-infected rhesus monkeys even if equal amounts of protein were given. A recombinant human alpha interferon with no antiviral activity on human cells in vitro proved very effective in the same in vivo system [37]. Also in rodent systems, discrepancies between the in vitro activity and the in vivo efficacy were reported [33, 41].

The obvious assumption is that these discrepancies between the in vitro and in vivo activity are caused by the other biological activities these interferons have. As with the anticancer activities in vitro, data on the sensitivity of viruses to interferon or the relative efficacy of different (sub)types in vitro cannot be extrapolated to the clinical situation.

In vivo activity of interferons against parasitic, bacterial and mycotic infections

Parasitic infections

Malaria is the most important parasitic disease in man. Hundreds of millions of people especially in the developing world are suffering from this disease. A number of chemotherapeutic agents are available as prophylaxis or therapy, but various *Plasmodium* strains are becoming increasingly resistant to chemotherapy. Neither is there a satisfactory treatment to eradicate the exo-erythrocytic forms of the parasites which are responsible for the chronicity of the infection.

In 1970 Jahiel and his coworkers reported that murine interferon alpha/beta in a dose of at least 1000 units injected 19, 22 and 26 h after infection of mice with *Plasmodium berghei* resulted in a longer incubation period, a reduced parasitemia and an increased survival[19].

Interferon gamma has also been shown to be active against different *Plasmodia* in rodents and in chimpanzees. Ferreira et al.[9] reported that as little as 150 units of recombinant murine interferon gamma, given intravenously 5 h before infection with *Plasmodium berghei*, was capable of reducing the parasitemia as monitored by Southern blotting. Five thousand (5000) units of recombinant rat interferon gamma reduced the amount of *Plasmodium berghei* (DNA) by more than 90%. In chimpanzees infected with *Plasmodium vivax* (which is one of the second major *Plasmodium* species infecting man) recombinant human interferon gamma reduced the parasitemia almost to zero.

Similar results have been reported by Maheshwari and coworkers in rhesus monkeys infected with *Plasmodium cynomolgi B.*[25]. The monkeys treated with 10^6 units of recombinant human interferon gamma per kg body weight intramusculary daily, from the day before infection until 13 days after, were free from blood parasites during the 60-day observation period. Lower doses induced less but still significant protection. The animals were only protected against sporozoite-induced infections and not to trophozoite-induced infections, suggesting that interferon inhibited the parasitic cycle in the hepatocyte only. Later the same group reported that the treatment period could be reduced to three daily injections of recombinant human gamma interferon starting the day before infection[31]. When the animals were reinfected with the parasite all treated animals were as sensitive to the reinfection as untreated controls, indicating that the interferon treatment had not resulted in immunity.

Clark et al. have studied the effect of recombinant murine interferon gamma in mice infected with the murine malaria *Plasmodium chabaudi*[6]. They treated the mice daily with 50 000 or 5000 units for seven days, starting the day before infection. Both doses delayed the onset of parasitemia in a dose-dependent manner. When the high dosage was given for 16 days the parasitemia was also significantly reduced. When these mice were rechallenged (33 days later) only the interferon-treated animals were found to be completely protected against the parasite.

The effect of recombinant murine interferon gamma in malaria caused by *Plasmodium vinckei*, as reported by Bienzle, was much less impressive[3]. Although the increase in survival time in mice prophylactically and/or therapeutically treated was statistically significant, the maximal increase attained was 2 days.

Toxoplasmic encephalitis is one of the more common opportunistic infections occurring in AIDS patients. The present therapeutic possibilities are unsatisfactory. McCabe and colleagues reported in 1984 that *Toxoplasma* was very sensitive to murine interferon gamma in vivo[27]. Five units injected every other day for 12 days had a significant effect, and 5000 units gave complete protection. Hofflin and Remington have reported synergy between roxithromycin and recombinant murine gamma interferon in a murine model for toxoplasmic encephalitis in which tachyzoite stages of the parasite are injected into the brain[16]. Roxithromycin treatment alone resulted in a survival rate of approximately 40%. When murine interferon gamma was added in a dose of 50 000 units given intravenously every other day for 4 days starting 6 h after infection, the survival rate increased to 100%.

There have also been reports on the efficacy in vivo of different interferon preparations against other parasitic infections; infection by *Trypanosoma cruzi*[20], a non-lethal intravenous infection with *Clamydia trachomatis*[45], and *Schistosoma mansoni* infection[29]. It is interesting to note that in the study of Pancré and colleagues the rat gamma interferon appeared to activate blood platelets, converting them into cytotoxic effector cells against the parasitic larvae, and also induced protective immunity to *S. mansoni*.

So, sensitivity to interferon treatment in vivo seems to be shared by many parasites. Up to now only one negative report on the efficacy of interferon in a parasitic disease has been published. Wyler et al.[44] failed to see an effect of murine interferon alpha/beta in mice infected with *Leishmania tropica*, although they established antiparasitic activity in the supernatants of stimulated spleen cells, suggesting that maybe gamma interferon may well be of interest in this disease[44].

In a murine model of a lethal pneumonia caused by *Chlamydia trachomatis*, recombinant murine interferon gamma only induced a significant protection in two of five experiments performed, although treatment with monoclonal antibodies to murine interferon gamma made the mice more sensitive to the infection[43]. The fact that nude mice were used may have contributed to the partial lack of effect.

The activity of interferon against bacterial and mycotic infections

The first report on the antibacterial activity of interferon preparations in experimental animals was published by Izadkhah and colleagues in 1980[17]. They showed that

murine serum containing interferon gamma in a dose of 5000 units a day was capable of protecting mice against a lethal challenge with *Salmonella typhimurium*. The interferon preparation they used was very impure, and was prepared by OT challenge of BCG immunized mice. This serum may, however, have contained a number of other cytokines apart from interferon gamma.

Purified murine interferon alpha/beta was reported to be active in infant mice against intragastric infection with *Salmonella typhimurium*, by Bukholm and coworkers in 1984[5]. Pretreatment of mice with 100 or 1000 units of this natural interferon resulted in a reduced mortality. The treatment could be postponed until 22 h after infection and remained effective. The mode of action in this infection seemed to be the inhibition of invasion of intestinal cells by this micro-organism rather than stimulation of the host defence mechanisms. Infant mice do indeed have an immature immune system which is not yet capable of responding to stimulation by interferon.

Pure recombinant murine interferon gamma was recently shown to be effective against *Klebsiella pneumoniae* infection of adult mice[15]. The mice were infected in the hind limbs with this organism, which is lethal in more than 90% of the animals. The experimental infection is considered to be a model for surgical wound infection. Recombinant murine interferon gamma in doses of 7500 and 750 units proved most effective when treatment was started 5 days before infection. A limited effect was noticed when the treatment was postponed until 1 h after infection.

The effect of recombinant murine interferon gamma has also been tested in a burn wound infection model in mice[14]. Mice treated with 7500 units subcutaneously daily during five days preceding the challenge of an artificial burn wound with *Klebsiella pneumoniae* survived significantly longer compared with controls. If *Pseudomonas aeruginosa* was used for the challenge, gamma interferon had no effect.

The effect of different rodent interferons on *Listeria monocytogenes* has also been studied. *Listeria monocytogenes* is a Gram-positive rod which is a facultative intracellular parasite of mononuclear and phagocytic cells. It is a common cause of neonatal sepsis and meningitis. Kiderlen et al.[21] showed that 1000 units of recombinant murine interferon gamma, injected the day before and on the day of infection with *Listeria* in the footpad of mice, resulted in a significant reduction of the number of bacteria that could be isolated. When the *Listeria* was injected intravenously 10^6 units had to be given to induce a significant reduction of bacteria.

Bortolussi and colleagues[4] have shown that both rat interferon alpha/beta as well as rat interferon gamma can protect 3-day-old rats against a lethal challenge with *Listeria monocytogenes*.

The rats had to be treated during three days, starting the day before infection. Interferon alpha/beta was active in doses of 10^5 and 10^3 units/kg body weight. Interferon gamma was only active in a dose of 10^5 units/kg body weight. The gamma interferon, however, induced a more significant reduction of bacterial count in the spleens than interferon alpha/beta.

In a subsequent study, the same group failed to find any effect of these interferon preparations when given after i.p. infection with *Listeria*. If the interferon was added to treatment of the animals with ampicilline, there was no additional beneficial effect. The bacterial count in the spleen was even increased in interferon-treated animals. The lack of effect of interferon given after infection was also noted in mice infected with *Mycobacterium tuberculosis*[22]. Although 1000–5000 units of recombinant murine interferon gamma given one day before infection with *Mycobacterium tubercolosis* reduced the number of micro-organisms in lungs and spleens, there was no effect when given 5 days after infection. There was no synergy with isoniazid therapy. So interferon seems only to be of limited usefulness in the treatment of bacterial infections, although some clinical applications are imaginable[24].

There is also one report on the in vivo efficacy of murine interferon alpha/beta in a mycotic infection[26]. Mice were treated with 20000 units intramusculary 6 h prior to intravenous infection with *Aspergillus fumigatus*. Interferon treatment resulted in a significant increase in the number of survivors. Interferon also had an effect in athymic mice, which suggests a role of the non-specific defence mechanisms such as NK cells or macrophages. In these mice, additional interferon treatments were given on the first and second days after infection.

Conclusions

Concepts about interferon have changed dramatically over the years. Initially it was considered to be an antiviral protein which selectively inhibited the replication of viruses[24]. Over the years we have discovered an increasing number of interferons and many different biological activities. Other regulatory proteins have been detected and the interferons have become part of an interacting family of biological response-modifying proteins. Because of the complexity of these systems, animal experiments are the only way to assess the clinical potential of interferons (and interferon-like molecules). It is important that the animal experiments should not be too restricted in scope, because interferon has now proved to have activity in conditions other than viral infections, for example against tumors and infections other than those caused by viruses.

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