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Recombinant interferon-gamma and chemotherapy with isoniazid and rifampicin in experimental murine tuberculosis

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Summary. Viable bacterial counts in the lungs and spleens of mice infected intravenously with *Mycobacterium tuberculosis*, strain H37Rv were reduced by intravenous recombinant murine interferon-γ (IFN-γ) 1000–5000 u, but not by 200 u. Reduction in counts was greatest when IFN-γ was given 1 day before infection and was not increased by additional doses in the preceding 2 days. The effect was complete in 1 day and was not increased by successive doses during the next week. Giving IFN-γ in multilamellar liposomes further reduced the spleen viable counts, but this appeared due to the liposomes themselves and not to encapsulation of IFN-γ within them. Only a minimal reduction in organ viable counts, not statistically significant, occurred when IFN-γ was given 5 days after infection. Although IFN-γ alone and isoniazid 25 mg/kg alone reduced the organ viable counts, combined treatment with IFN-γ and isoniazid was no more bactericidal than isoniazid alone. Similarly, the bactericidal activity of rifampicin 25 mg/kg was not increased by simultaneous administration of IFN-γ. There seems little likelihood that IFN-γ would increase the efficacy of the early stages of the chemotherapy of tuberculosis.

Keywords: murine tuberculosis, interferon-gamma, liposomes, isoniazid, rifampicin

Interferon-gamma (IFN-γ) is a potent lymphokine (Trinchieri & Perussia 1985), increasing the microbicidal (Kiderlen *et al.* 1984; McCabe *et al.* 1984) and tumouricidal (Fleischmann *et al.* 1980) activity in experimental murine models. It is a glycosylated protein produced by T lymphocytes and is one of the most important immunoregulatory molecules. Recently, recombinant human (Gray *et al.* 1982) and murine (Gray & Goeddel 1983) IFN-γs have been obtained by cloning the relevant genes in *Escherichia coli.* While there is evidence that IFN-γ is protective in several viral diseases (Baron *et al.* 1982; Degre & Rollag 1982; Preble & Friedman 1983), less is known about bacterial diseases. Activity would be expected to be greatest against intracellular bacteria in which macrophage function may well be a determining factor in immunity. Treatment with IFN-γ before infection has been found to protect mice against *Salmonella typhimurium* (Izadkhah *et al.* 1980) and *Listeria monocytogenes* (Kiderlen *et al.* 1984). There have been no reports of the effect of IFN-γ in experimental infections with virulent *Mycobacterium tuberculosis.*

One of the ways that immunomodulators

might be used is to increase the antibacterial action of chemotherapeutic drugs during treatment. A potent immunomodulator, which increases macrophage function, might increase the bactericidal activity of the drug in a manner analogous to the synergism or additive effects when two bactericidal drugs are used together (Jawetz & Gunnison 1952). Alternatively, if it only inhibited growth, it might reduce the bactericidal action of the drug, as occurs when a bacteriostatic drug such as chloramphenicol is used with a bactericidal drug such as penicillin G in the treatment of bacterial infections (Garrod *et al.* 1981). We wished to explore the interaction between IFN-γ and the two drugs isoniazid (H) and rifampicin (R) usually considered to be the most effective of those available for the chemotherapy of murine and human tuberculosis.

When pure recombinant IFN-γ became available in quantities sufficient for extensive study in the mouse, we undertook an examination of its effects alone and in combination with H and R in experimental murine tuberculosis. The results obtained in a model of acute disease are reported here.

Materials and methods

Media. All media were obtained from Difco Laboratories (PO Box 14B, Central Avenue, West Molesey, Surrey). Selective 7H11 agar (Mitchison *et al.* 1973) was prepared by the addition of polymyxin B 200 units/ml, carbenicillin 100 mg/l, trimethoprim 20 mg/l and amphotericin B 10 mg/l to Middlebrook 7H11 agar supplemented with 10% oleic acid albumin dextrose complex. Middlebrook 7H9 Tween-albumin liquid medium was supplemented with 10% albumin dextrose complex.

Mycobacteria. The virulent H37Rv strain of *M. tuberculosis* was obained from the Trudeau Mycobacterial Culture Collection (TMC 102). The strain was passaged in the mouse, grown in 7H9 medium and stored at $-70°C$ (Kim & Kubica 1972) before inoculation into mice.

Interferon. Escherichia coli-derived recombinant murine IFN-γ, produced by Genentech Inc., was supplied by Messrs Boehringer Ingelheim (Vienna, Austria). It was diluted in phosphate buffered saline (PBS) containing homologous mouse serum 1 mg total protein/ml immediately before being injected intravenously in 0.1 ml volumes, or in 0.2 ml volumes when in liposomes.

Chemotherapeutic drugs. Rifampicin (Ciba Laboratories, Horsham, West Sussex) was suspended in 0.2% methyl cellulose containing 0.05% Tween 80. Isoniazid (Sigma Chemical Co., Poole, Dorset) was dissolved in distilled water. Both drugs were administered in 0.2 ml volumes to mice by oral gavage.

Liposomes. Multilamellar vesicle (MLV) liposomes were prepared by a modification of the method of Fidler (1980). A mixture of L-α-phosphatidylcholine (PC) and L-α-phosphatidyl-L-serine (PS) in a PC:PS ratio of 7:3 moles was evaporated to dryness in a vacuum rotary evaporator. The lipid film was then hydrated with the material to be encapsulated for 30 min before being vortex mixed for 5 min. The resultant liposome preparation contained 2.5 μmole lipid in the 0.2 ml volume injected into mice.

Infections of mice. Specific pathogen free BALB/c mice (18–20 g weight) were randomly allocated into experimental groups. The frozen bottles of *M. tuberculosis* in 7H9 medium were rapidly thawed, diluted with an equal volume of 0.1% sterile gelatine in normal saline and sonicated with a probe sonicator (Rinco Ultrasonics UK, Ltd, PO Box 217, London) for 15 s before being injected intravenously in 0.2 ml volumes.

Counts of viable bacilli in the organs. Spleens and lungs were removed aseptically into sterile hard glass homogenizer tubes and

diluent, consisting of 2% bovine serum albumin (BSA) in normal saline, was added to make up the volume to 5 ml. After homogenization with a motor-drive polytetrafluorethylene pestle (Pierce *et al.* 1953), 10-fold dilutions were made in 0.1% BSA and 0.1 ml aliquots were placed on one-third segments of selective 7H11 plates. The plates were packed into polythene bags and incubated for 3–4 weeks at 37°C before the colonies were counted.

Statistical analysis. The results of all experiments were examined by 2-way and 3-way factorial analysis of variance using the statistical computer packages MINITAB and GLIM.

Results

Effect of treatment with IFN-γ before infection

The effect of dose size of IFN-γ given before and during an acute infection are shown in Fig. 1. In neither the lungs nor the spleen did 200 units of IFN-γ have a detectable effect on the viable counts, whereas 1000 u and 5000 u reduced the counts to a similar extent at days 3, 5 and 9 by 1.8 times in the lungs and by 1.4 times in the spleen, on the average. Analysis of variance showed a higher mean square for replicate error in the lungs (0.112) than in the spleen (0.009), a constant feature of all experiments, so that small differences in counts were more often statistically significant in the spleen. The reduction of counts by IFN-γ was significant ($P<0.01$) in the spleen but not in the lungs ($P>0.05$).

Fig. 2 shows the effect of giving 1000 u IFN-γ in solution or in liposomes before and during an acute infection. The replicate error mean square was 0.042 in the lung counts and 0.009 in the spleen counts. In the lungs, delivery in liposomes, whether of PBS or IFN-γ, had no apparent effect on the viable counts. However, in the spleen, counts in animals receiving liposomes were on the average 1.4 times lower than the corresponding counts on those not given liposomes, whether PBS or IFN-γ was given. The liposome main effect in the analysis of variance was highly significant ($P<0.001$) while the IFN-γ × liposome interaction was small (0.001) and not significant, indicating that liposomes increased spleen immunity,

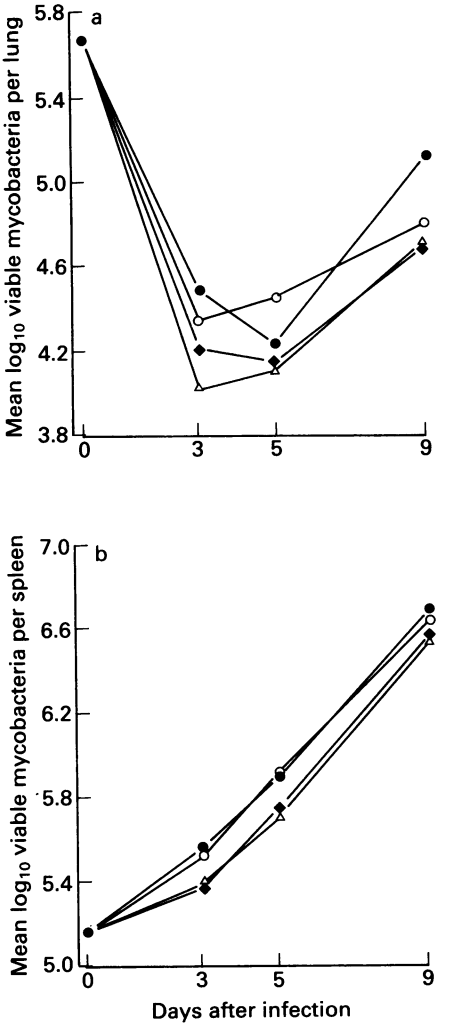

Fig. 1. Effect of dose size of interferon on the growth of *M. tuberculosis* in mouse organs. Mice were each infected with 1.3×10^6 viable *M. tuberculosis* on day 0 and treated with PBS containing 0 u (●), 200 u (○), 1000 u (♦) or 5000 u (△) IFN-γ on days −2, +1, +4, and +7. Each point represents the mean $\log_{10}$ cfu per organ of five mice.

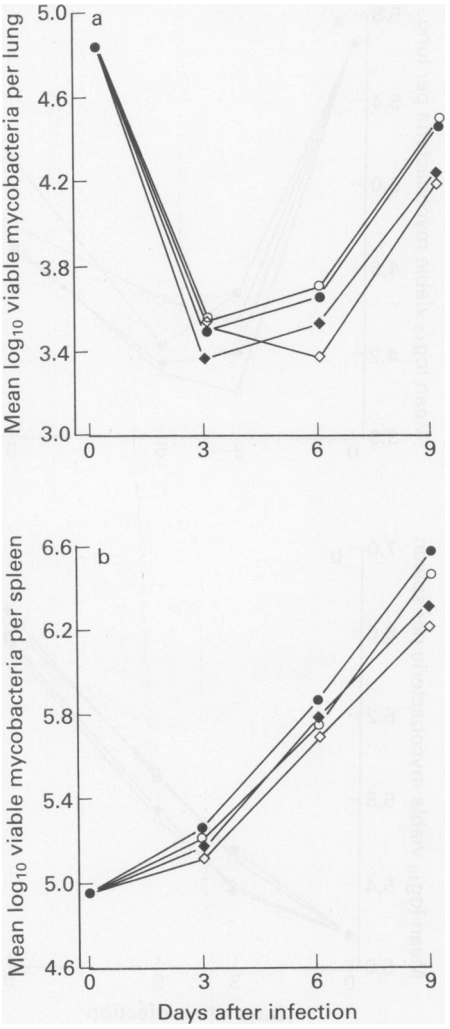

Fig. 2. Effect of administration of interferon in liposomes on the viable counts of *M. tuberculosis* in mouse organs. Mice were each infected with 7.5×10^5 viable organisms on day 0 and treated with PBS (●), PBS in liposomes (○), 1000 u IFN-γ (♦) or 1000 u IFN-γ in liposomes (◊) on days -2, $+1$, $+4$ and $+7$. Each point represents the mean $\log_{10}$ cfu per organ of four mice.

irrespective of their contents. Taking the mean of the results with and without liposomes, counts at 3, 6 and 9 days were decreased by 1.4 in the lung ($P<0.01$) and by 1.5 in the spleen ($P<0.001$) as a result of administration of 1000 u IFN-γ. In summary, host immunity was increased to a small extent in the spleen only by liposomes and to a larger extent in both organs by IFN-γ, but the specific effect of IFN-γ was not altered by giving it in liposomes.

In the two experiments considered previously (Figs 1 and 2) and in other similar experiments not illustrated here, it was evident that the effect of IFN-γ was established early, within 3 days after infection, and that the viable count curves from the organs of IFN-γ-treated mice and their controls remained approximately parallel thereafter. Furthermore, no statistically significant interaction between IFN-γ dosage and day of the experiment was ever encountered in their analyses of variance. Hence, it seemed likely that the IFN-γ dose given 2 days before infection, rather than the 3 doses given after infection, was responsible for most or all of the IFN-γ effect. The effects of the timing and the duration of pretreatment with doses of 2000 u IFN-γ were examined in an experiment lasting only 2 days. Viable counts from the organs of six mice given three preinfection doses of IFN-γ and from six control mice not given IFN-γ were set up at 1 h after infection. The mean $\log_{10}$ counts from IFN-γ and control lungs were 5.02 and 5.00, respectively, and from their spleens 4.78 and 4.70, respectively, neither difference attaining statistical significance. Having thus established that IFN-γ pretreatment did not affect the phagocytosis of the infecting inoculum of *M. tuberculosis*, organ counts were done at 1 and 2 days after infection (Fig. 3). In the lungs, no significant differences were found between any of the rhythms of administering IFN-γ pretreatment, the replicate error mean square being as usual large (0.360). In the spleen, however, highly significant differences were found ($P<0.001$), as the replicate error mean square was only 0.010. The largest difference from the control mice not given IFN-γ was found in the groups given one dose of IFN-γ 1 day before infection, three doses 1, 2 and 3 days beforehand, and two doses 0 and 3 days beforehand. A smaller difference was

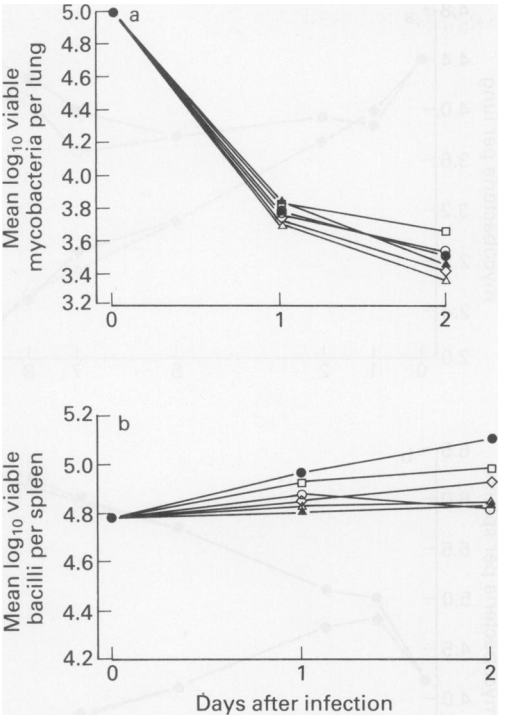

Fig. 3. Effects of timing and multiplicity doses with interferon on viable counts of *M. tuberculosis* in mouse organs. Mice were pretreated with one dose of 2000 u IFN-γ on day -3 (□), day -2 (◊), or day -1 (○), two doses of 2000 u IFN-γ (△), three doses of 2000 u IFN-γ (▲), or received PBS only (●); they were infected with 4.1×10^5 viable organisms on day 0. Each point represents the mean $\log_{10}$ cfu per organ of four mice.

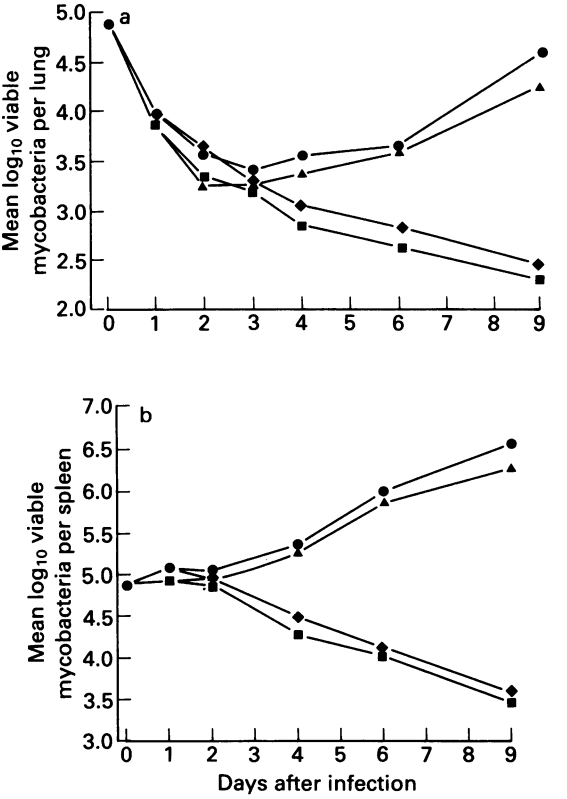

Fig. 4. Effect of interferon and isoniazid alone and in combination on the growth of *M. tuberculosis* in mouse organs when interferon was administered before and after infection. Mice were each infected with 6.6×10^5 viable organisms and given, IFN-γ (▲) on days -2, $+1$, $+4$ and $+7$, daily isoniazid (◊) from day $+1$, IFN-γ on days -2, $+1$, $+4$, $+7$ with daily isoniazid from day $+1$ (■), or received PBS only (●). IFN-γ was used at 2000 u per mouse and isoniazid at 25 mg/kg. Each point represents the mean $\log_{10}$ cfu per organ of four mice.

found in mice given one dose 2 days beforehand and the least difference was in those given a single dose 3 days beforehand. Thus the greatest effect in increasing immunity was seen if the interval between the pretreatment dose of IFN-γ and infection was only 1 day and this effect was not increased by giving multiple doses over the preceding period.

Interaction of IFN-γ and chemotherapy with H or R

Figure 4 shows an experiment in which IFN-γ was administered in the usual pattern with doses of 2000 u given 2 days before infection and then at 1, 4 and 7 days after infection, and H (25 mg/kg body weight) was given daily, starting 1 day after infection. At day 1, the counts from IFN-γ-treated mice were lower than those from the control mice, both in lungs and spleens. From day 2 onwards, the curves for the mice receiving H diverged from the conrols. The curves for the lungs and spleens of mice given IFN-γ as well as H were lower than for those given H alone, but the difference remained constant during the

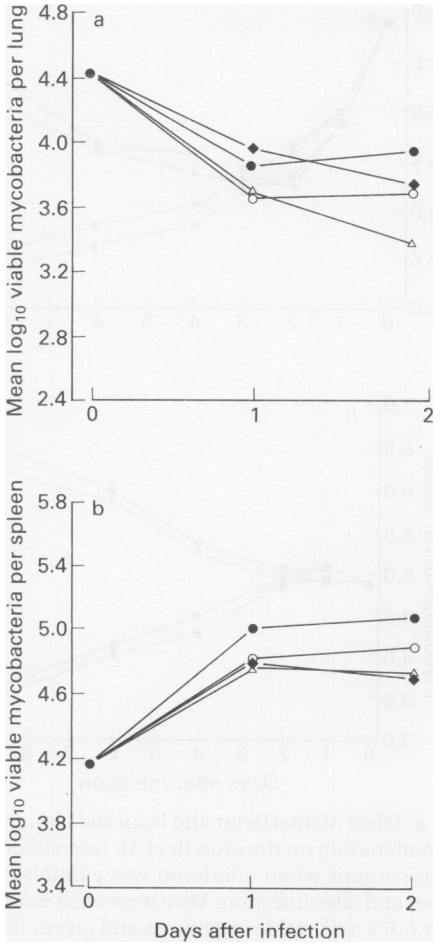

Fig. 5. Effect of interferon and isoniazid alone and in combination on the growth of *M. tuberculosis* in mouse organs during the first 2 days after infection. Mice were each infected with 3×10^5 viable organisms on day 0 and given IFN-γ (○) on days -1 and $+1$, daily isoniazid (◊) from day 0, IFN-γ on days -1, $+1$ with isoniazid from day 0 (△), or were given PBS only (●). IFN-γ was given at 2000 u per mouse and isoniazid at 25 mg/kg. Each point represents the mean $\log_{10}$ cfu per organ of four mice.

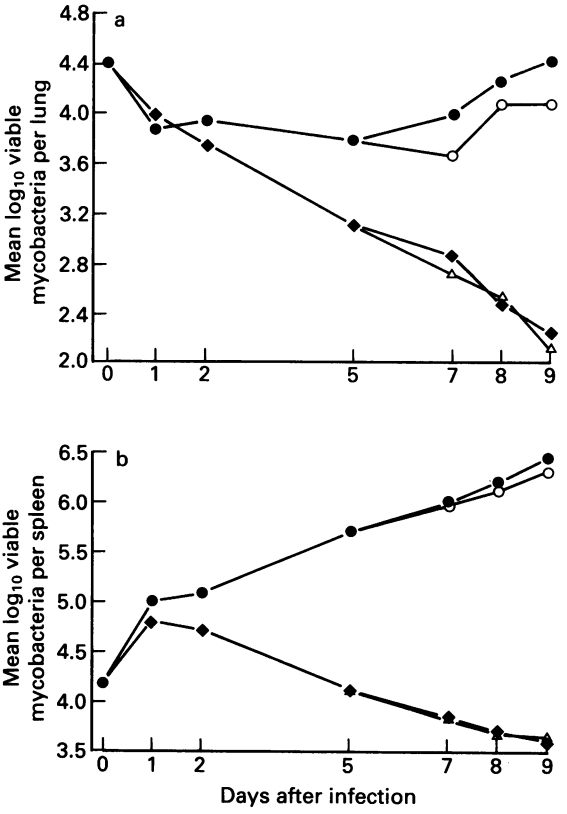

Fig. 6. Effect of interferon and isoniazid alone and in combination on the growth of *M. tuberculosis* in mouse organs. IFN-γ was administered on days $+5$ and $+7$ and isoniazid from day 0. Mice were each infected with 3×10^5 viable organisms on day 0 and given IFN-γ (○) on days $+5$ and $+7$, daily isoniazid (◊) from day 0, IFN-γ on days $+5$ and $+7$ with daily isoniazid from day 0 (△), or were given PBS only (●). IFN-γ was given at 2000 u per mouse and isoniazid at 25 mg/kg. Each point represents the mean $\log_{10}$ cfu per organ of four mice.

experiment and was similar in extent to the difference attributable to IFN-γ at day 1. It seems that the apparent additive bactericidal effect between IFN-γ and H was entirely due to the initial effect of IFN-γ during the first day *before* H was given.

In a second more complex experiment, H was started as early as possible, about 1 h after infection and was given daily for 9 days. IFN-γ was given to some of the mice (Group 1) 1 day before and 1 day after infection and to further mice (Group 2), which had been receiving H from the start, 5 and 7 days after infection. Mice from group 1 (Fig. 5) were killed at day 1 and day 2, insufficiently long

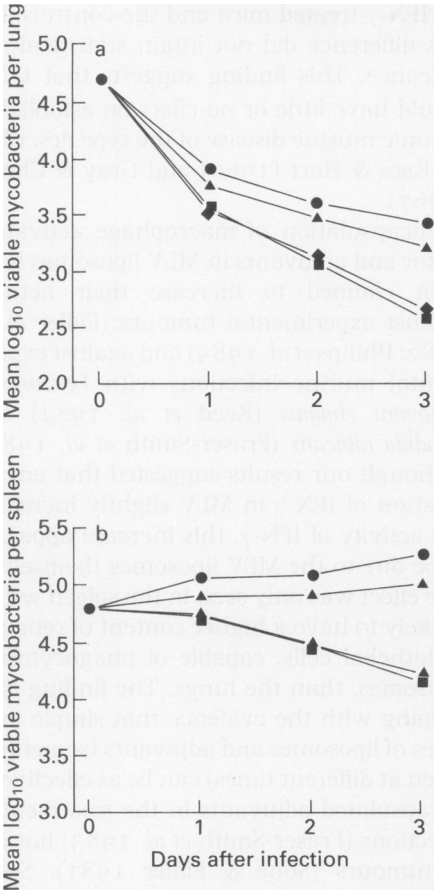

Fig. 7. Effect of interferon and rifampicin alone and in combination on the growth of *M. tuberculosis* in mouse organs. Mice were each infected with 4.8×10^5 viable organisms and given IFN-γ (▲) on days -1 and $+1$, daily rifampicin (♦) from day 0, IFN-γ on days -2 and $+1$ with daily rifampicin from day 0 (■), or were given PBS only (●). IFN-γ was given at 2000 u per mouse and rifampicin at 25 mg/kg. Each point represents the mean $\log_{10}$ cfu per organ of four mice.

for the action of H to be fully evident. In the lungs, IFN-γ had a mean effect (averaged over days 1 and 2 and compared to control and IFN-γ only groups) that was only just significant ($P<0.05$) but the mean effect of H in the factorial design did not attain significance. No conclusions can therefore be drawn about the interaction of IFN-γ and H in the lungs. However, in the spleen counts (which had much smaller replicate error), the IFN-γ alone counts appeared lower than the control counts, as did the H alone counts, but the curve for H+IFN-γ was almost identical to that for H alone. The absence of any additive effect between IFN-γ and H was confirmed by significant interaction ($P<0.01$) between the main effects of H and IFN-γ in the analysis of variance. The results in the Group 2 mice (Fig. 6) during the 4 days after IFN-γ was first given on day 5, show an apparent small decrease in the lung counts and an even smaller decrease in the spleen counts of mice given IFN-γ as compared to the control untreated mice, though neither effect attains statistical significance. H continued to be bactericidal during the period, but this bactericidal action was not modified by IFN-γ. In summary, IFN-γ had no effect on the bactericidal activity of H during the first 2 days of infection. It had little or no real effect by itself in an established infection from 5 days onwards, not did it alter the bactericidal action of H over the period.

Fig. 7 shows the counts obtained in an experiment in which mice were pretreated with 2000 u IFN-γ and R (25 mg/kg) was given on the day of infection and on the next 2 days. IFN-γ alone had the usual small effect evident from day 1 onwards in lungs and spleens. The bactericidal activity of R was rapid from day 1 to the end of the experiment. IFN-γ had no effect on the bactericidal activity of R since the curves for R alone and IFN-γ+R were closely similar in the lungs and spleens. The analysis of variance of the results from the lung counts indicated a highly significant effect of R ($P<0.001$), but a barely significant effect of IFN-γ ($P<0.05$); the IFN-$\gamma \times$ R interaction was not significant. In the spleen results, bactericidal indifference between the effects of IFN-γ and R was confirmed by the large interaction between the IFN-γ and the R effects in the analysis of variance ($P<0.01$).

Discussion

These findings clearly demonstrate that IFN-γ had a small, but reproducible effect in reducing the organ counts of *M. tuberculosis* in acute experimental murine tuberculosis. Consistent results could only be obtained by using specific pathogen free mice bred under particularly clean conditions. We report elsewhere (Khor *et al.* 1986) that peritoneal macrophages were activated *in vitro* by IFN-γ for bacteriostatic and bactericidal activity against *Listeria monocytogenes* and *Mycobacterium microti.* Hence, it is reasonable to assume that the increased immunity seen in the whole mouse model was due to the activation of macrophages, as is often the case in intracellular infections. Banerjee *et al.* (1986) have also found that recombinant murine IFN-γ given before or at the same time as infection of mice with *Mycobacterium bovis* (BCG) reduced the viable bacterial count in the spleen. There is little reason to suppose that the other expressions of IFN-γ, namely its enhancement of class II MHC and its anti-proliferative activity (Trinchieri & Perussia 1985), influenced the model of acute murine tuberculosis that we have studied.

The administration of different dose sizes suggested that 200 u IFN-γ had a hardly detectable effect, while doses of 1000–5000 u were equally and fully effective. The main effect was produced by doses given before infection, multiple pre-infection doses being no better than single doses; the optimal interval appeared to be about 1 day before infection. Additional dosage after infection probably did not influence immunity, since almost all of the difference between organ counts of treated and control mice occurred in the first day after infection. Preinfection dosage with IFN-γ has been shown to be protective in murine toxoplasmosis by McCabe *et al.* (1984) and in murine listeriosis by Kiderlen *et al.* (1984). In the experiment in which IFN-γ was started 5 days after infection, there was a small apparent difference between the subsequent organ counts for IFN-γ-treated mice and the controls, but this difference did not attain statistical significance. This finding suggests that IFN-γ would have little or no effect on established chronic murine disease of the type described by Rees & Hart (1960) and Gray & Cheers (1967).

Encapsulation of macrophage activating factor and adjuvants in MLV liposomes have been claimed to increase their activity against experimental tumours (Fidler *et al.* 1982; Philips *et al.* 1985) and against experimental murine infections with *Leishmania donovani chagasi* (Reed *et al.* 1984) and *Candida albicans* (Fraser-Smith *et al.* 1983). Although our results suggested that encapsulation of IFN-γ in MLV slightly increased the activity of IFN-γ, this increase appeared to be due to the MLV liposomes themselves. The effect was only seen in the spleen which is likely to have a higher content of reticuloendothelial cells, capable of phagocytosing liposomes, than the lungs. The finding is in keeping with the evidence that simple mixtures of liposomes and adjuvants (sometimes given at different times) can be as effective as encapsulated adjuvants in the treatment of infections (Fraser-Smith *et al.* 1983) but not of tumours (Sone & Fidler 1981). Some activity of liposomes themselves is evident, though often not statistically significant, from the controls used in several studies of adjuvant action against infections and tumours (Fraser-Smith *et al.* 1983; Koff *et al.* 1985).

IFN-γ given alone and the antibacterial drugs isoniazid and rifampicin given alone could each reduce the organ counts consistently. However, the effect of giving both together never exceeded that of the antibacterial drug alone. It is relevant that although corticotrophin and corticosteroids promoted the growth of *M. tuberculosis* in experimental murine tuberculosis, these hormones did not alter the bactericidal activity of isoniazid and pyrazinamide (Batten & McCune 1957*a*,*b*). Mitchison & Selkon (1956) showed that isoniazid was more bactericidal in guinea pigs previously BCG vaccinated than in

unvaccinated animals and that the effect continued and may have increased for several weeks after infection. However, the effect of BCG alone on the organ counts was much greater and more prolonged than the effect of IFN-γ. The explanation for these findings might lie in the much higher potency of antibacterial drugs than of lymphokine (or steroids) in killing (or promoting the growth of) bacteria. Perhaps all bacteria whose growth is influenced by lymphokine or steroids are inhibited or killed by the antibacterial drug. Only when a highly effective modifier of the immune response such as BCG was used was its antibacterial potency (perhaps measured as the proportion of macrophages modified) even nearly as great as that of isoniazid so that there may not have been equivalence between the bacterial populations under the control of the immune response and the drug. Relevant to the possible use of IFN-γ to improve chemotherapy is our finding that it had little effect when given to mice 5 days after infection, when macrophages would have been likely to have been activated by the infection itself. We conclude that there is little likelihood of IFN-γ influencing the early stages of the chemotherapy of established tuberculosis.

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References

BANERJEE D.K., SHARP A.K. & LOWRIE D.B. (1986) The effect of gamma-interferon during *Mycobacterium bovis* (BCG) infection in athymic and euthymic mice. *Microb. Pathog.* **1**, 221–224.

BARON S., HOWIE V., LANGFORD M., MACDONALD E.M., STANTON G.J., REIMYER J. & WEIGERT D.A. (1982) Induction of interferon by bacteria, protozoa and viruses: defensive role. *Tex. Rep. Biol. Med.* **41**, 150–157.

BATEN J.C. & MCCUNE R.M. (1957*a*) The influence of corticotrophin and certain steroids on populations of *Mycobacterium tuberculosis* in tissues of mice. *Br. J. exp. Path.* **38**, 413–423.

BATTEN J.C. & MCCUNE R.M. (1957*b*) The influence of corticotrophin and cortisone with antituberculous drugs on populations of *Mycobacterium tuberculosis* in tissues of mice. *Br. J. exp. Path.* **38**, 424–437.

DEGRE M. & ROLLAG H. (1982) On the possible role of interferon in infections with non-viral agents. *Tex. Rep. Biol. Med.* **41**, 388–94.

FIDLER I.J. (1980) Therapy of spontaneous metastases intravenous injection of liposomes containing lymphokine. *Science* **208**, 1469–1471.

FIDLER I.J., BARNES Z.L., FOGLER W.E., KIRSH R., BUGELSKI P. & POSTE G. (1982) Involvement of macrophages in the eradication of established metastases following intravenous injection of liposomes containing macrophage activators. *Cancer Res.* **40**, 496–501.

FLEISCHMAN W.R., KLEYN K.M. & BARON S. (1980) Potentiation of antitumour effect of virus-induced interferon by mouse immune interferon preparations. *J. natl. Cancer Inst.* **65**, 963–966.

FRASER-SMITH E.B., EPPSTEIN D.A., LARSEN M. & MATTHEWS T.R. (1983) Protective effect of a muramyl dipeptide analog encapsulated in or mixed with liposomes against *Candida albicans* infection. *Infect. Immun.* **39**, 172–178.

GARROD L.P., LAMBERT H.P. & O'GRADY F. (1981) *Antibiotic and chemotherapy*. pp 276–277. Edinburgh: Churchill Livingstone.

GRAY D.F. & CHEERS C. (1967) The steady state in cellular immunity. I. Chemotherapy and superinfection in murine tuberculosis. *Aust. J. exp. Biol. Med. Sci.* **45**, 407–416.

GRAY P.W. & GOEDDEL D.V. (1983) Cloning and expression of murine immune interferon cDNA. *Proc. Natl. Acad. Sci.* **80**, 5842–5846.

GRAY P.W., LEUNG D.W., PENNICA D., YELVERTON E., NAJARIAN R., SIMONSEN C.C., DERYNK R., SHERWOOD P.J., WALLACE D.M., BERGER S.L., LEVINSON A.D. & GOEDDEL D.V. (1982) Expression of human immune interferon cDNA in *Escherichia coli* and monkey cells. *Nature* **295**, 503–508.

IZADKHAH Z., MANDEL A.D. & SONNENFELD G. (1980) Effect of treatment of mice with sera containing gamma interferon on the course of infection with Salmonella typhimurium strain LT-2. *J. Interferon Res.* **1**, 137–145.

JAWETZ E. & GUNNISON J.B. (1952) Studies on

antibiotic synergism and antagonism: scheme of combined antibiotic action. *Antibiot. Chemother.* **2**, 243–248.

KHOR S.Y., LOWRIE D.B. & MITCHISON D.A. (1986) Effects of recombinant interferon-gamma and chemotherapy with isoniazid and rifampicin on infections of mouse peritoneal macrophages with *Listeria monocytogenes* and *Mycobacterium tuberculosis* in vitro. *Br. J exp. Path.* in press.

KIDERLEN A.F., KAUFMANN S.H.E. & LOHMANN-MATTHES M-L. (1984) Protection of mice against the intracellular bacterium *Listeria monocytogenes* by recombinant immune interferon. *Eur. J. Immun.* **14**, 964–967.

KIM T.H. & KUBICA G.P. (1973) Preservation of mycobacteria: 100% viability of suspensions stored at −70°C. *Appl. Microbiol.* **25**, 956–960.

KOFF W.C., SHOWALTER S.D., HAMPAR B. & FIDLER I.J. (1985) Protection of mice against fatal herpes simplex type 2 infection by liposomes containing muramyl tripeptide. *Science* **228**, 495–496.

MCCABE R.E., LUFT B.J. & REMINGTON J.S. (1984) Effect of murine interferon gamma on murine toxoplasmosis. *J. infec. Dis.* **150**, 961–967.

MITCHISON D.A., ALLEN B.W., CARROL L., DICKINSON J.M. & ABER V.R. (1972) A selective oleic acid albumin agar medium for tubercle bacilli. *J. med. Microbiol.* **5**, 165–172.

MITCHISON D.A. & SELKON J.B. (1956) The bactericidal activities of antituberculous drugs. *Am. Rev. Tuberc. pulm. Dis.* **74**, Suppl., 109–116.

PHILIPS N.C., MORAS M.L., CHEDID L., LEFRANCIER P. & BERNARD J.M. (1985) Activation of alveolar macrophage tumoricidal activity and eradication of experimental metastases by freeze-dried liposomes containing a new lipophilic muramyl dipeptide derivative. *Cancer Res.* **45**, 128–134.

PIERCE C.H., DUBOS R.J. & SCHAEFFER W.B. (1953) Multiplication and survival of tubercle bacilli in the organs of mice. *J. exp. Med.* **97**, 189–205.

PREBLE O.T. & FRIEDMAN R.M. (1983) Biology of disease. Interferon-induced alterations in cells: relevance to viral and nonviral diseases. *Lab. Invest.* **49**, 4–18.

REED S.G., BARRAL-NETTO M. & INVERSO J.A. (1984) Treatment of experimental visceral leishmaniasis with lymphokine encapsulated in liposomes. *J. Immun.* **132**, 3116–3119.

REES R.J.W. & HART P.D. (1960) Analysis of the host-parasite equilibrium in chronic murine tuberculosis by total and viable bacillary counts. *Br. J. exp. Path.* **42**, 83–88.

SONE S. & FIDLER I.J. (1981) *In vitro* activation of tumoricidal properties in rat alveolar macrophages by synthetic muramyl dipeptide encapsulation in liposomes. *Cell. Immun.* **57**, 42–50.

TRINCHIERI G. & PERUSSIA B. (1985) Immune interferon: a pleiotropic lymphokine with multiple effects. *Immun. Today* **6**, 131–136.