INFECTION AND IMMUNITY, Feb. 1991, p. 580–586
0019-9567/91/020580-07$02.00/0

Vol. 59, No. 2

Gamma Interferon-Mediated Inhibition of *Eimeria vermiformis* Growth in Cultured Fibroblasts and Epithelial Cells

M. ELAINE ROSE,[1]* A. L. SMITH,[1] AND D. WAKELIN[2]

Houghton Laboratory, Institute for Animal Health, Houghton, Huntingdon, Cambridgeshire PE17 2DA,[1] and Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD,[2] United Kingdom

Received 2 August 1990/Accepted 2 November 1990

The growth of *Eimeria vermiformis* within cultured murine fibroblastlike (L-929) or rat epitheliallike (RATEC) cells was inhibited by treatment of the cells with the appropriate recombinant gamma interferon. The effect was apparent as a reduction in both the initial numbers of intracellular sporozoites and, to a much greater extent, the numbers of subsequent developmental stages. Pretreatment of the host cells was more effective than treatment in the early postinvasive period, and recombinant gamma interferon had no effect on the development of the parasite if added 24 h or later after the inoculation of sporozoites. Incubation of sporozoites in medium containing recombinant gamma interferon in no way affected their ability to invade or to grow within host cells. These findings indicate that the inhibitory effects of recombinant gamma interferon on the growth of *E. vermiformis* are mediated via the host cell and are directed mainly against the transforming sporozoite, although the ability of the sporozoite to invade the host cell was also reduced to some extent. The later developmental stages were refractory to the effects of this lymphokine.

Gamma interferon (IFN-γ) has been shown to play an important part in the defense of the host against several intracellular protozoan diseases; for example, malaria (8, 14, 31, 32), leishmaniasis (18, 19, 28), Chagas' disease (23), and toxoplasmosis (15, 17, 35). It is also implicated in the control of coccidiosis, an economically important disease of domestic animals, especially poultry, caused by protozoa of the genus *Eimeria*, which parasitize enterocytes. In a murine model system, depletion of endogenous IFN-γ by treatment with specific neutralizing monoclonal antibodies led to severe, often fatal, infections with *Eimeria vermiformis* in a strain of mouse that is normally resistant to this species (27) and greatly increased the severity of the clinical signs produced by infection with *Eimeria pragensis* in BALB/c, NIH, and C57BL/6 mice (27a). As a component of the cytokine network, IFN-γ has a major immunomodulating activity, regulating the cells of the immune system and the expression of cell surface antigens, including those of the major histocompatibility complex; it also has cytostatic and antimicrobial effects (4, 36). Either or both of these major types of function may operate against intracellular protozoa, and a knowledge of their relative importance, in the case of any particular organism, is necessary for devising rationally based means for control by vaccination. In the case of leishmania (19) and *Trypanosoma cruzi* (23), exogenous IFN-γ has been shown to activate macrophages for antimicrobial activity, whereas it can act more directly against plasmodia by inhibiting the extraerythrocytic developmental stages within hepatocytes (8, 16, 31). In the case of toxoplasma, IFN-γ is thought to be effective both by activation of macrophages (35) and by inhibition of growth within cells that are not immune effectors (22). Inhibition of the development of *Eimeria* spp. within cultured monocytes or macrophages by crude supernatants of both specific antigen- and concanavalin A-stimulated lymphocytes has been described (9, 20, 34), but in vivo the activation of professional phagocytes may not be of major importance since the host cell is normally an enterocyte.

More recently, Kogut and Lange (11, 12) found that pretreatment of cultured kidney or epidermoid carcinoma cells with crude supernatants from concanavalin A-stimulated T lymphocytes or with appropriate recombinant IFN-γ (rIFN-γ) led to reduced invasion by eimerian sporozoites and inhibition of their development. Additionally, but surprisingly, Lillehoj et al. (13) reported decreased growth of *Eimeria tenella* in a bovine kidney cell line (MDBK) that had been pretreated with crude supernatants from nonspecifically or specifically stimulated chicken T cells or from a cultured Marek's disease virus-transformed, chicken-derived, T-cell line. Since macrophages were not involved in either case, the antiparasite effect of IFN-γ clearly was mediated via the host cell.

In this report we describe the effects of murine (mrIFN-γ) or rat (rrIFN-γ) rIFN-γ on the development of *E. vermiformis* in cultured murine fibroblasts (L-929 cells) or rat epithelial cells (RATEC cells) and show that (i) both invasion and growth of the parasite are inhibited, and (ii) the antidevelopmental effects of the lymphokine are directed against the newly invaded sporozoite and later developmental stages are unaffected.

MATERIALS AND METHODS

Parasites. The origin and methods of propagation of *E. vermiformis* have been described elsewhere (26). Sporozoites were excysted from mechanically broken (21) oocysts by incubation in trypsin-bile solution, as recommended for *E. tenella* by Chapman (6), and purified by passage through DE-52 cellulose (30).

Reagents. mrIFN-γ was kindly provided by G. R. Adolf, Ernst-Boehringer (Vienna, Austria) or obtained from Holland Biotechnology (Leiden, The Netherlands); rrIFN-γ, more than 99% pure, was produced from CHO cells in which the chromosomal gene of rat IFN-γ was cloned (7) (Holland Biotechnology). rIFN-γ was reconstituted and stored in

* Corresponding author.

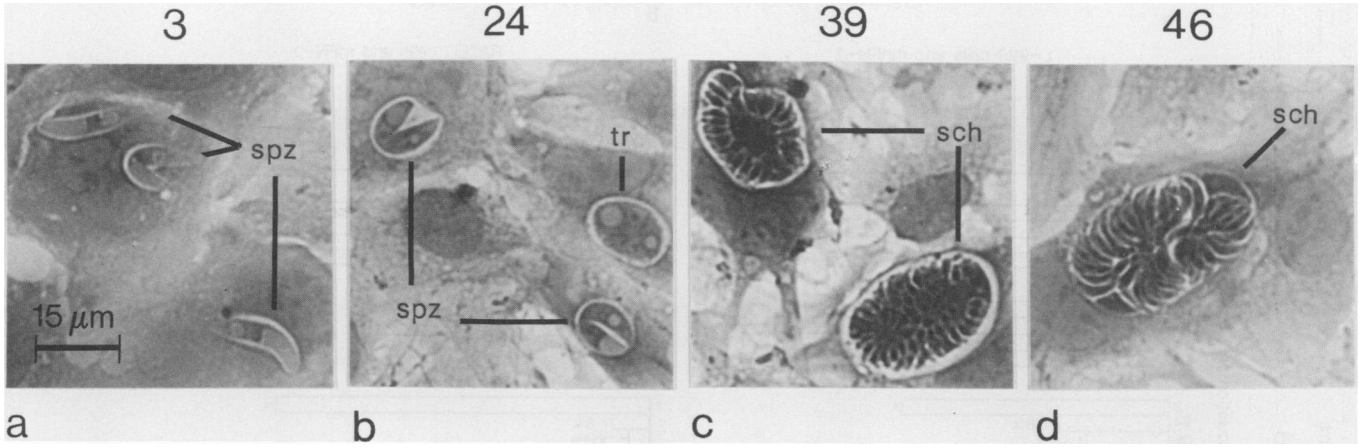

FIG. 1. Developmental stages of *E. vermiformis* in rat epithelial cells. (a) Newly invaded sporozoites (spz); (b) sporozoites and a trophozoite (tr); (c) Immature schizonts (sch); (d) mature schizont. Numbers above panels indicate hours after inoculation with sporozoites.

accordance with the instructions of the suppliers and incorporated in the culture medium as indicated in Results.

Cultivation of *E. vermiformis* in vitro. Murine fibroblastlike L-929 cells (Flow Laboratories, Rickmansworth, United Kingdom) were maintained in Eagle minimal essential medium containing 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (50 μg/ml), 1% nonessential amino acids, and 10% inactivated fetal calf serum (FCS). RATEC cells (29) (Flow Laboratories, Irvine, Scotland) were cultured in Dulbecco modified minimal essential medium containing 2 mM L-glutamine, antibiotics, and 10% FCS. For the culture of *E. vermiformis*, suspended L-929 cells (5×10^6/ml) were treated with 25 μg of mitomycin C per ml for 30 min at 37°C, washed three times with FCS-free medium, and inoculated (1×10^6 cells in 2 ml of medium containing 5% FCS) onto grease-free glass coverslips (18 by 18 mm), placed in 35-mm-diameter plastic tissue culture dishes (Nunc, Gibco, Uxbridge, United Kingdom), and incubated overnight. Treatment with mitomycin C to prevent proliferation was not necessary with the RATEC cell line. For infection, the medium was replaced by a suspension of sporozoites (2.2×10^5 in 2 ml per dish) in medium containing 1% FCS (inoculation medium). After 3 h of incubation, to allow invasion, the medium was removed, the dishes were rinsed three times, and medium containing 5% FCS (2.5% FCS for RATEC cells) was added. All incubations were at 37°C in humidified 95% air–5% CO_2.

Assessment of growth of *E. vermiformis*. Coverslips were sampled for examination with a microscope at approximately 3, 24, 36, and 45 h after the addition of sporozoites, fixed in formal saline, and stained with hematoxylin and eosin. The numbers of intracellular parasites present and their stages of development were determined at a magnification of ×400. Each treatment was set up in duplicate, and at least 20 fields were examined on each coverslip. The results are expressed as numbers of intracellular parasites per field and percentages of intracellular parasites that have transformed from sporozoites (trophozoites or immature or mature schizonts, according to the time of sampling). Comparisons between treatments were analyzed by Student's *t* test.

RESULTS

Growth of *E. vermiformis* in vitro. A proportion of sporozoites invaded cells quickly; the numbers increased with time of incubation up to 2 h, after which they remained virtually unchanged (see Fig. 5), even though extracellular sporozoites were found throughout the early periods of incubation; 3 h was taken as a standard time for sampling for invasion. Many sporozoites assumed a V or U shape shortly after invasion and were surrounded by a parasitophorous vacuole. In L-929 cells at 24 h, enlarged, well-defined sporozoites were present, and approximately 30% had become trophozoites. It was difficult to differentiate between this developmental stage and early immature schizonts. At 36 h, more than 90% of the parasites seen were schizonts and up to 25% of these were fully formed. Mature schizonts were in the majority (60 to 70%) at 45 h, and there were numerous extracellular and intracellular merozoites. No further development occurred. The numbers of parasites that developed in control, untreated dishes varied between but not within experiments, from approximately 5 to 16 per field (14 to 44% of sporozoites inoculated). Development in RATEC cells was slower (14% trophozoites at 24 h; the ratio of immature to mature schizonts was approximately 1:1 at 45 h), and the parasites tended to be larger than those in L-929 cells. The developmental stages are illustrated in Fig. 1.

Effect of mrIFN-γ on growth of *E. vermiformis* in cultured L-929 cells. In preliminary dose-response experiments, the protocol for treatment with rIFN-γ was similar to that used for investigations involving plasmodia (31); i.e., cells were pretreated with rIFN-γ for 6 h, dishes were washed, and sporozoites were inoculated (time 0 h) and allowed to invade for 3 h, at which time the inoculation medium was replaced with mrIFN-γ-containing (test) medium. Incubation was continued for up to 45 h after inoculation, with removal and replacement of test medium at 24 and 36 h. Control dishes were similarly treated with IFN-free medium. The results obtained with concentrations of mrIFN-γ ranging from 1 to 100 U/ml of culture medium and L-929 cells are given in Fig. 2a. mrIFN-γ at 1 U/ml had no significant effect on the invasion or growth of the parasites, but mrIFN-γ at 10, 50, or 100 U/ml had a profound effect upon growth and a slight but significant ($P = 0.03$ for 10 U/ml, $P = 0.04$ for 100 U/ml) effect upon invasion. Interestingly, the development of the small numbers of parasites present at 36 or 45 h on coverslips treated with 10 to 100 U of mrIFN-γ per ml was no different from that of controls, and the parasites were morphologically normal. At 24 h there was an indication that the development of parasites in cells subjected to these concen-

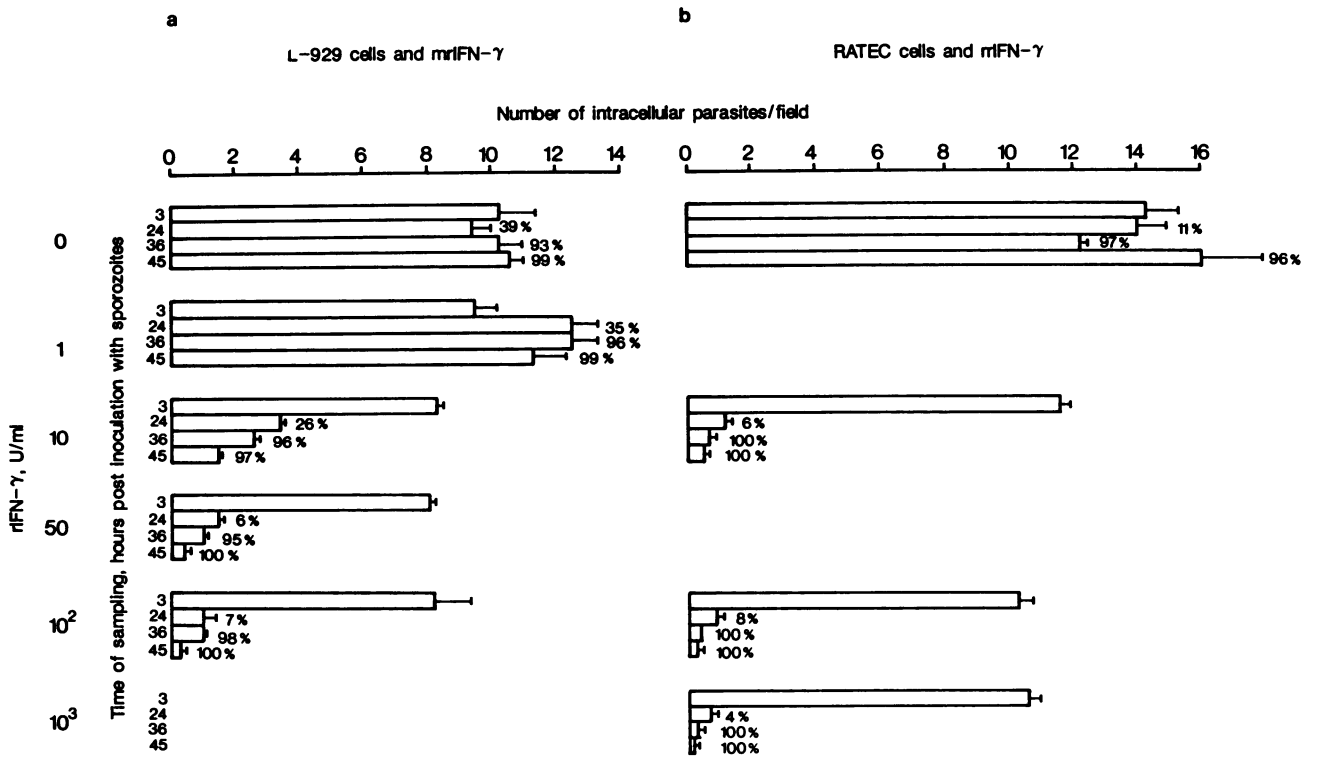

FIG. 2. Invasion and growth of *E. vermiformis* in cultured cells: effects of treatment of host cells with rIFN-γ. (a) L-929 (murine fibroblastlike) cells and mrIFN-γ. (b) RATEC (rat epitheliallike) cells and rrIFN-γ. Monolayers were pretreated with rIFN-γ (test medium) from 0 to 6 h and then washed. Sporozoites were inoculated in IFN-γ-free medium and allowed to invade for 3 h, after which the monolayers were washed and the test medium was replaced at this and other times (24, 36, and 45 h postinoculation) of sampling. The numbers of parasites that were developing are given as percentages of the numbers present.

trations of mrIFN-γ lagged behind that seen in the control coverslips or in coverslips treated with 1 U of mrIFN-γ per ml. However, this comparison is of doubtful validity, since development is incomplete and variable at this time and the numbers of intracellular parasites present in the treated coverslips are low. These results were confirmed in other experiments that included dishes treated with 1,000 U of IFN-γ per ml (data not shown). The findings obtained with RATEC cells treated with rrIFN-γ were very similar (Fig. 1b). A standard dose of 100 U of rIFN-γ per ml was adopted in subsequent experiments.

Effect of pretreatment of sporozoites of *E. vermiformis* with mrIFN-γ. To determine whether pretreatment of sporozoites could affect their ability to invade L-929 cells, they were incubated at 10^6/ml in FCS-free medium containing 100 U of mrIFN-γ per ml for 30 min at 37°C; after sporozoites were washed to remove mrIFN-γ, they were inoculated onto L-929 cells and cultured for up to 45 h. The results obtained with coverslips sampled at 3, 24, 36, and 45 h did not differ from those found with control coverslips from dishes that had been inoculated with sporozoites preincubated in mrIFN-γ-free medium (Fig. 3).

Effect of timing of treatment of host cells with rIFN-γ on growth of *E. vermiformis*: developmental stage affected. The effects of varying the length of pretreatment of L-929 cells and of application of mrIFN-γ after the invasion of sporozoites are shown in Fig. 4. Included as known positive controls were dishes treated as in the dose-response experiment described above (Fig. 2); i.e., cells were pretreated from −6 to 0 h, and rIFN-γ was renewed at each time of sampling (multiple application).

As in the dose-response experiments, two types of effect were apparent: first, a reduction in the initial number of intracellular parasites, i.e., sporozoites (sampled at 3 h), and, second, a further reduction in the numbers of subsequent developmental stages (sampled at 24, 36, and 45 h). There was no significant effect on the development of the

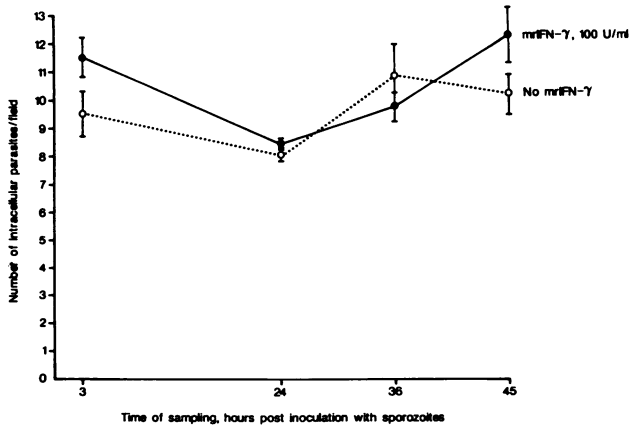

FIG. 3. Invasion and growth of *E. vermiformis* in cultured L-929 cells: effect of pretreatment of sporozoites with mrIFN-γ. Sporozoites (10^6/ml) were incubated in serum-free medium containing 100 U of mrIFN-γ per ml for 30 min, washed, and inoculated into the cultures at 0 h. Suspensions similarly incubated in IFN-free medium were used as controls.

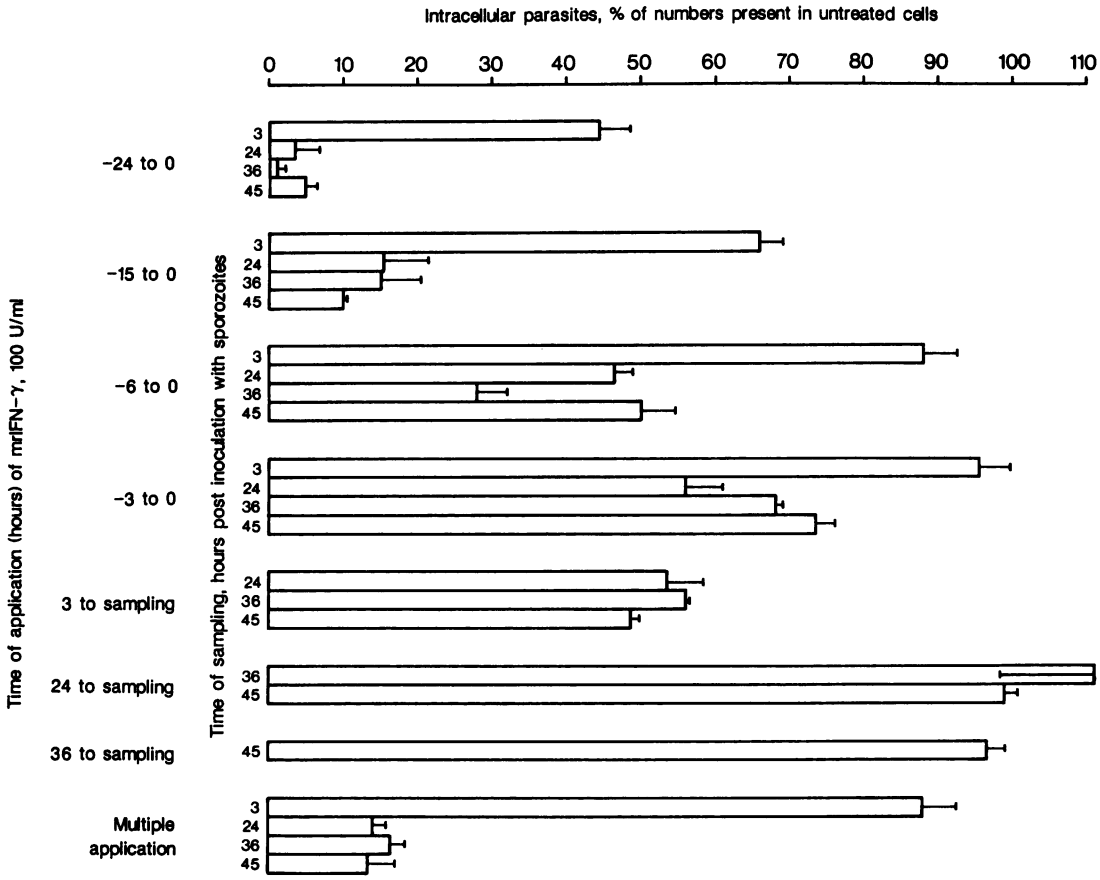

FIG. 4. Effect of differing treatments with mrIFN-γ on invasion and growth of *E. vermiformis* in cultured L-929 cells. Monolayers were treated with mrIFN-γ (100 U/ml) for the times indicated. For the multiple application, monolayers were pretreated from −6 to 0 h, and rIFN-γ was renewed at each time of sampling, i.e., at 3, 24, and 36 h. Sporozoites in IFN-γ free medium were inoculated at 0 h.

parasites that persisted. The effect on the initial numbers of intracellular sporozoites (invasion) varied directly with the length of pretreatment between −24 to 0 h and −6 to 0 h ($P \leq 0.013$); the difference between −6 to 0 h and −3 to 0 h was not significant. Reduction in the numbers of subsequent intracellular parasites was also directly related to the length of pretreatment and, in the case of 24-h pretreatment, was equivalent to the inhibition found with the standard treatment (i.e., pretreatment for 6 h with renewal from 3 h until time of sampling). Posttreatment from 3 h postinoculation had the effect of halving the number of subsequent stages, but, if delayed until 24 or 36 h postinoculation, it was ineffective; there were no differences between the numbers of intracellular parasites found at 36 and 45 h or at 45 h, respectively. Thus, intracellular parasites after invasion must have been affected within the first 24 h. Similar results were obtained with RATEC cells and rrIFN-γ (data not shown).

Since pretreatment of the host cells caused a reduction in the numbers of intracellular sporozoites present at 3 h, it was necessary to determine whether this effect was uniformly exerted throughout the period allowed for invasion (0 to 3 h) or became apparent at a particular time point. The kinetics of invasion did not appear to be altered as a result of IFN-γ treatment of the host cells (Fig. 5a). In comparison with controls, smaller numbers of sporozoites were present within pretreated cells, but their rate of accumulation did not differ markedly. Subsequent growth in the cultures was assessed at 24, 36, and 45 h with results similar to those obtained previously, i.e., marked reduction, in comparison with controls, of numbers of intracellular parasites in treated dishes at 24 h and no major change at 36 or 45 h (data not shown).

Figure 5b gives the results of a similar experiment, which included cultures pretreated from −48 to −24 h, washed to remove mrIFN-γ, and incubated in normal medium for 24 h before the inoculation of sporozoites. The numbers of sporozoites that invaded these cells were significantly lower ($P = 0.0001$) than those in untreated cultures, indicating that the host cells had not recovered from treatment in the course of 24 h of incubation in IFN-free medium.

The effects of treatment of L-929 cells with mrIFN-γ on the critical postinvasion period between 3 and 24 h are shown in Fig. 6. As expected, there were significantly fewer ($P \leq 0.005$) parasites in treated monolayers than in control cells at all times of sampling. In the IFN-treated cells there was a marked decline in the numbers of intracellular parasites within the first 9 h after invasion ($P = 0.003$ and $P = 0.001$ for differences between 3 and 6 h and between 6 and 9 h, respectively), after which the numbers of parasites fell gradually until 45 h. In the untreated cells, on the other hand, there was no significant change between successive samplings, but, unusually, fewer parasites were present at 45 h than at 3 h ($P = 0.001$). The results confirm the deleterious effect of IFN early in development and indicate that it is most marked within the first 12 h of incubation. We could

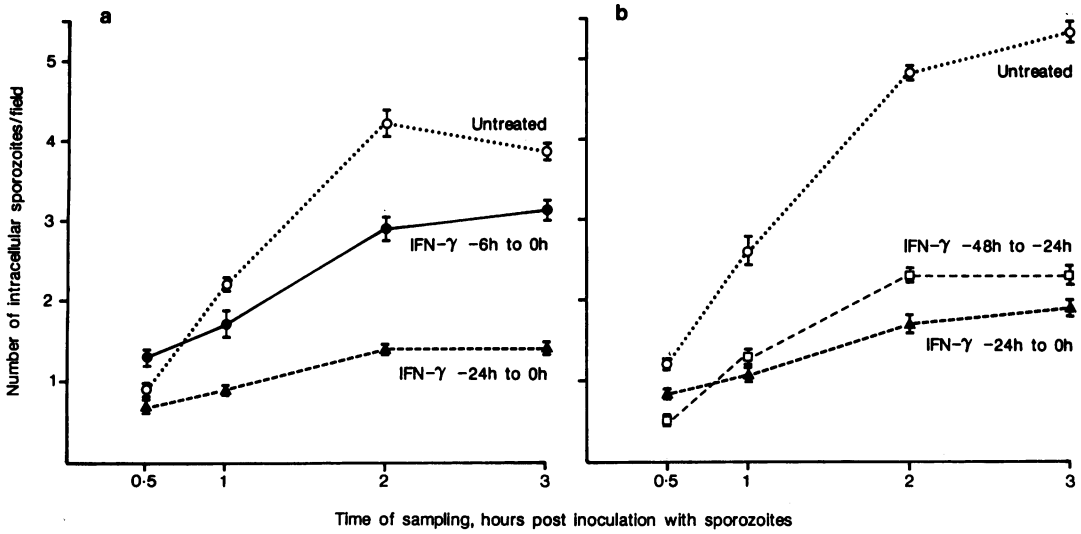

FIG. 5. Invasion of L-929 cells by sporozoites of *E. vermiformis*: effect of pretreatment of host cells with 100 U of mrIFN-γ per ml. (a) Monolayers were pretreated for 6 h (●) up to the time (0 h) of inoculation of freshly excysted sporozoites in IFN-γ-free medium. (b) Pretreatment for 24 h was followed by incubation for 24 h in IFN-γ-free medium before the inoculation of sporozoites (□). (a and b) Monolayers were untreated (○) or treated for 24 h up to the time of inoculation of sporozoites (▽).

not, however, identify with certainty sporozoites in any stage of degeneration.

DISCUSSION

The development of *E. vermiformis* in cultured L-929 or RATEC cells was very similar to that described by Kelley and Youssef (10) in cultures of primary whole mouse embryo cells or MDBK cells. The life cycle proceeded normally up to and including the entry of cells by merozoites liberated from first-generation schizonts, but there was no further growth. The system therefore provided a means for studying, in the absence of the modulating effects produced by cells of the immunologic system, interactions between IFN-γ and parasites in the initial invasive stages (sporozoites) and completion of the development of the first-generation schizont in host cells that are not part of the immunologic system.

The ability of sporozoites to invade and subsequently grow within host cells was not affected by preliminary incubation in mrIFN-γ, confirming results obtained for *E. tenella* and bovine rIFN-γ (11, 12). Similarly, human rIFN-γ (hrIFN-γ) had no effect on the viability of extracellular tachyzoites of *Toxoplasma gondii* (22).

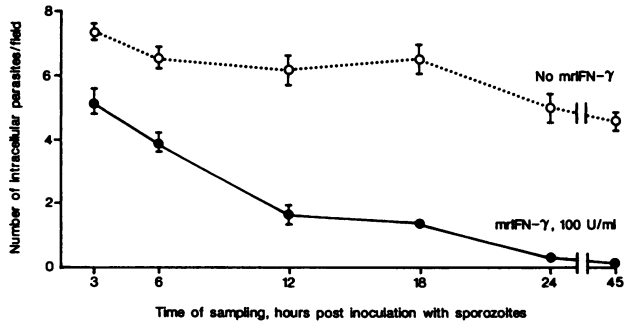

FIG. 6. Effect of pretreatment of L-929 host cells with mrIFN-γ on numbers of intracellular parasites. Symbols: ●, 100 U of mrIFN-γ per ml included in incubation medium for 24 h before the inoculation of sporozoites in IFN-γ-free medium at 0 h; ○, untreated cells.

Treatment of host monolayers with rIFN-γ, however, had two effects upon the host-parasite relationship: a reduction in the initial numbers of sporozoites within cells (at 3 h) and, to a far greater extent, a reduction in the numbers of parasites present at 24, 35, or 48 h. Those parasites that were present at and after 24 h, however, developed normally.

Inhibition of invasion by sporozoites (of *E. tenella*) of cultured host cells as a result of pretreatment of the monolayers by IFN-γ has also been described by Kogut and Lange (11), who examined interactions in MDBK and human epidermoid (HEp-2) cells with bovine rIFN-γ and hrIFN-γ, respectively. Their results differed from ours and between their two systems only in the length of treatment and the amount of rIFN-γ required to produce the effect. The inhibitory effect of IFN-γ on invasion by eimerian sporozoites is therefore now well established. The design of similar experiments with plasmodia, human hepatoma cells, and hrIFN-γ (8, 16, 31) or *T. gondii*, human fibroblasts, and hrIFN-γ (22) did not allow this aspect of the inhibitory effects of IFN-γ to be investigated, since cultures were examined only for growth of the parasites. However, Mellouk et al. (16) suggested that inhibition of the development of *Plasmodium falciparum* was the result of mechanisms that were active only after penetration. The quantitative aspects of an effect on invasion by other eimerian developmental stages (i.e., merozoites) could not be examined in our system due to the limitation of growth to the first generation of schizogony.

The mechanisms of invasion and inhibition are not known, but, since sporozoites were not affected directly and the effect increased with the length of pretreatment, it is likely that the mechanisms involve alterations in the surface membrane of the host cell. IFN-γ exerts a multiplicity of effects on a variety of cells (36) by binding to their membranes and altering the expression of cell surface receptors (5). The molecular aspects of cellular invasion by eimerian sporozo-

ites are little understood, but perturbations of the host cell surface are known to inhibit invasion and there is some evidence for a nonspecific, immune-induced inhibition of invasion in vivo (1), which could be due to the action of IFN-γ.

Inhibition of invasion in vitro was but a minor component of the total antiparasite effect, as has been demonstrated in immunized hosts in vivo (2, 24, 25). The major effect of treatment with IFN-γ, which was both dose (Fig. 2) and exposure time (Fig. 4) dependent, was evident as a greatly decreased number of parasites at 12 h and subsequent times of sampling. This suggests that the transforming sporozoite could be the target of attack, a hypothesis that was supported by the lack of effect of rIFN-γ when added to cultures at 24 h or later, i.e., after the sporozoites had begun to transform. It is possible that subsequent invasive forms, i.e., merozoites, could also be affected at this stage, but it was not possible to examine this in our system. Interestingly, the few parasites that survived in cells pretreated or treated at an early stage with rIFN-γ appeared normal and had developed to the same extent as those within control, untreated host cells. In contrast, the development of the reduced numbers of *E. tenella* present in bovine rIFN-γ-treated MDBK cells was reported to be inhibited, with "less than 30% of the parasites present developing beyond the sporozoite stage," and the undeveloped sporozoites were "normal looking as judged by light microscopy" (12). The reason for these discrepancies is not immediately apparent but may reflect differences in the species of parasite and the host cell. That such differences may be relevant is evident from previous work. Thus, Speer et al. (34) reported that sporozoites of *Eimeria bovis*, whose development in bovine monocytes had been inhibited by pretreatment of the host cells with concanavalin A-stimulated lymphocyte supernatants, appeared structurally normal, whereas sporozoites of *Eimeria papillata* examined in the same system were destroyed intracellularly. Also, Schwartzman et al. (33), while confirming the inhibition of growth of *T. gondii* in human fibroblasts pretreated with hrIFN-γ (22), found that similar treatment of murine fibroblasts (L-929 cells) with mrIFN-γ had no such effect. This was attributed to the inability of the L-929 cells to produce indoleamine 2,3-dioxygenase, which degrades tryptophan, in response to treatment. This finding suggests that tryptophan starvation was not the cause of the reduction in numbers of eimeria observed by us in mrIFN-γ-treated L-929 cells. Tryptophan starvation has also been discounted as being responsible for the antimalarial activity of hrIFN-γ in cultured hepatocytes (31). We cannot speculate on the means by which treatment with IFN-γ affects the transformation of eimerian sporozoites because this process is little understood, although it does seem to involve the metabolism of glucose (37).

Some of the mechanisms responsible for the antiviral effects induced by IFN treatment are known, but these may differ from those which inhibit cell proliferation by altering intracellular metabolism (3); either or both types could be responsible for intracellular antieimeria activity. It is unlikely that a nonspecific adverse effect of IFN-γ on the host cells was responsible for our findings, since the monolayers retained their integrity throughout incubation and the antieimeria effects were apparent only against the initial development of the parasite. Normal development to mature first-generation schizonts of the very small minority of parasites that escaped the IFN-γ-mediated effects could be due to a lack of response to the lymphokine by that particular host cell.

In vivo, the antieimeria effects of IFN-γ are likely to be exerted in a variety of ways, not the least of which will be mediated via the cells of the immune system. In addition to these immune-mediated effects, the results described here indicate that the lymphokine is capable of exerting a more direct antiparasite effect, manifest by (i) rendering the host cell less susceptible to invasion and (ii) inducing intracellular changes that adversely affect the transformation of the sporozoites (and possibly other invasive stages) and bring about their destruction. That these effects were demonstrable in vitro, not only with highly IFN-sensitive L-929 fibroblastlike cells (which are not hosts to eimeria in vivo) but also with RATEC epithelial cells, suggests that such mechanisms could operate in vivo in infected animals.

ACKNOWLEDGMENTS

Brian Millard took the photomicrographs, Stuart Hodgson drew the figures, and Elaine Collier provided the typescript.

The work was supported by grants (13425 and 031118) from the Wellcome Trust.

REFERENCES

1. **Augustine, P. C.** 1989. The *Eimeria*: cellular invasion and host-cell parasite interactions, p. 205–215. *In* P. Yvoré (ed.), Coccidia and intestinal coccidiomorphs, Proceedings of the Vth International Coccidiosis Conference, 1989. no. 49. Les Colloques de l'Institut National de la Recherche Agronomique, Institut National de la Recherche Agronomique, Paris.
2. **Augustine, P. C., and H. D. Danforth.** 1986. A study of the dynamics of the invasion of immunized birds by *Eimeria* sporozoites. Avian Dis. **30**:347–351.
3. **Baglioni, C.** 1985. Mechanisms of action of interferons, p. 53–56. *In* W. J. Streilean et al. (ed.), Advances in gene technology: molecular biology of the immune system. Proceedings of the 17th Miami Winter Symposium. International Council of Scientific Unions Short Reports, vol. 2. Cambridge University Press, Cambridge.
4. **Balkwill, F. R., and F. Burke.** 1989. The cytokine network. Immunol. Today **10**:299–304.
5. **Chang, E. H., E. F. Grollman, F. T. Jay, G. Lee, L. D. Kohn, and R. M. Friedman.** 1978. Membrane alterations following interferon treatment. Adv. Exp. Med. Biol. **110**:85–99.
6. **Chapman, H. D.** 1978. Studies on the excystation of different species of *Eimeria* in vitro. Z. Parasitenkd. **56**:115–121.
7. **Dijkema, R., P. H. van der Meide, P. H. Pouwels, M. Caspers, M. Dubbeld, and H. Schellekens.** 1985. Cloning and expression of the chromosomal immune interferon gene of the rat. EMBO J. **4**:761–767.
8. **Ferreira, A., L. Schofield, V. Enea, H. Schellekens, P. van der Meide, W. E. Collins, R. S. Nussenzweig, and V. Nussenzweig.** 1986. Inhibition of development of exoerythrocytic forms of malaria parasites by γ-interferon. Science **232**:881–884.
9. **Hughes, H. P. A., C. A. Speer, J. E. Kyle, and J. P. Dubey.** 1987. Activation of murine macrophages and a bovine monocyte cell line by bovine lymphokines to kill the intracellular pathogens *Eimeria bovis* and *Toxoplasma gondii*. Infect. Immun. **55**:784–791.
10. **Kelley, G. L., and N. N. Youssef.** 1977. Development in cell cultures of *Eimeria vermiformis* Ernst, Chobotar and Hammond, 1971. Z. Parasitenkd. **53**:23–29.
11. **Kogut, M., and C. Lange.** 1989. Recombinant interferon-γ inhibits cell invasion by *Eimeria tenella*. J. Interferon Res. **9**:67–77.
12. **Kogut, M., and C. Lange.** 1989. Interferon-γ mediated inhibition of the development of *Eimeria tenella* in cultured cells. J. Parasitol. **75**:313–317.
13. **Lillehoj, H. S., S. Y. Kang, L. Keller, and M. Sevoian.** 1989. *Eimeria tenella* and *E. acervulina*: lymphokines secreted by an avian T cell against avian coccidiosis. Exp. Parasitol. **69**:54–64.
14. **Maheshwari, R. K., C. W. Czarniecki, G. P. Dutta, S. K. Puri, B. N. Dhawan, and R. M. Friedman.** 1986. Recombinant human

gamma interferon inhibits simian malaria. Infect. Immun. **53:** 628–630.

15. **McCabe, R. E., B. J. Luft, and J. S. Remington.** 1984. Effect of murine interferon gamma on murine toxoplasmosis. J. Infect. Dis. **150:**961–962.
16. **Mellouk, S., R. K. Maheshwari, A. Rhodes-Feuillette, R. L. Beaudoin, N. Berbiguier, H. Matile, F. Miltgen, I. Landau, S. Pied, J. P. Chigot, R. M. Friedman, and D. Maxier.** 1987. Inhibitory activity of interferons and interleukin 1 on the development of *Plasmodium falciparum* in human hepatocyte cultures. J. Immunol. **139:**4192–4195.
17. **Murray, H. W., B. Y. Rubin, and C. D. Rothermel.** 1983. Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes. Evidence that interferon-γ is the activating lymphokine. J. Clin. Invest. **72:**1506–1510.
18. **Murray, H. W., G. L. Spitalny, and C. F. Nathan.** 1985. Activation of mouse peritoneal macrophages *in vitro* and *in vivo* by interferon-γ. J. Immunol. **134:**1619–1622.
19. **Nacy, C. A., M. Belosevic, M. S. Meltzer, and D. L. Hoover.** 1988. Leishmania, p. 167–191. *In* G. I. Byrne and J. Turco (ed.), Interferon and non-viral pathogens. Marcel Dekker, Inc., New York.
20. **Onaga, H., M. Tajima, and T. Ishii.** 1983. Activation of macrophages by culture fluid of antigen-stimulated spleen cells collected from chickens immunized with *Eimeria tenella*. Vet. Parasitol. **13:**1–11.
21. **Patton, W. H.** 1965. *Eimeria tenella*: cultivation of the asexual stages in cultured animal cells. Science **150:**767–769.
22. **Pfefferkorn, E. R., and P. M. Guyre.** 1984. Inhibition of growth of *Toxoplasma gondii* in cultured fibroblasts by human recombinant gamma interferon. Infect. Immun. **44:**211–216.
23. **Reed, S. G.** 1988. *In vivo* administration of recombinant IFN-γ induces macrophage activation, and prevents acute disease, immune suppression, and death in experimental *Trypanosoma cruzi* infections. J. Immunol. **140:**4342–4347.
24. **Riley, D., and M. A. Fernando.** 1988. *Eimeria maxima* (Apicomplexa): a comparison of sporozoite transport in naive and immune chickens. J. Parasitol. **74:**103–110.
25. **Rose, M. E., A. M. Lawn, and B. J. Millard.** 1984. The effect of immunity on the early events in the life-cycle of *Eimeria tenella* in the caecal mucosa of the chicken. Parasitology **88:**199–210.
26. **Rose, M. E., D. G. Owen, and P. Hesketh.** 1984. Susceptibility to coccidiosis: effect of strain of mouse on reproduction of *Eimeria vermiformis*. Parasitology **88:**45–54.
27. **Rose, M. E., D. Wakelin, and P. Hesketh.** 1989. Gamma interferon controls *Eimeria vermiformis* primary infection in BALB/c mice. Infect. Immun. **57:**1599–1603.

27a. **Rose, M. E., D. Wakelin, and P. Hesketh.** Parasite Immunol., in press.

28. **Sadick, M. D., R. M. Locksley, C. Tubbs, and H. V. Raff.** 1986. Murine cutaneous leishmaniasis: resistance correlates with the capacity to generate interferon-γ in response to leishmania antigens in vitro. J. Immunol. **136:**655–661.
29. **Schellekens, H., G. A. de Wilde, and W. Weimar.** 1980. Production and initial characterization of rat interferon. J. Gen. Virol. **46:**243–247.
30. **Schmatz, D. M., M. S. J. Crane, and P. K. Murray.** 1984. Purification of *Eimeria* sporozoites by DE-52 anion exchange chromatography. J. Protozool. **31:**181–183.
31. **Schofield, L., A. Ferreira, R. Altszuler, V. Nussenzweig, and R. S. Nussenzweig.** 1987. Interferon-γ inhibits the intrahepatocytic development of malaria parasites *in vitro*. J. Immunol. **139:**2020–2025.
32. **Schofield, L., J. Villaquiran, A. Ferreira, H. Schellekens, R. Nussenzweig, and V. Nussenzweig.** 1987. γ interferon, $CD8^+$ T cells and antibodies required for immunity to malaria. Nature (London) **330:**664–666.
33. **Schwartzman, J. D., S. L. Gonias, and E. R. Pfefferkorn.** 1990. Murine gamma interferon fails to inhibit *Toxoplasma gondii* growth in murine fibroblasts. Infect. Immun. **58:**833–834.
34. **Speer, C. A., D. W. Reduker, D. E. Burgess, W. M. Whitmire, and G. A. Splitter.** 1985. Lymphokine-induced inhibition of growth of *Eimeria bovis* and *Eimeria papillata* (Apicomplexa) in cultured bovine monocytes. Infect. Immun. **50:**566–571.
35. **Suzuki, Y., M. A. Orellano, R. D. Schreiber, and J. S. Remington.** 1988. Interferon-γ: the major mediator of resistance against *Toxoplasma gondii*. Science **240:**516–518.
36. **Trinchieri, G., and B. Perussia.** 1985. Immune interferon: a pleiotropic lymphokine with multiple effects. Immunol. Today **6:**131–136.
37. **Wang, C. C.** 1982. Biochemistry and physiology of coccidia, p. 167–228. *In* P. L. Long (ed.), The biology of the coccidia. University Park Press, Baltimore.