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Effect of Recombinant Human Gamma Interferon on Intracellular Activities of Antibiotics against *Listeria monocytogenes* in the Human Macrophage Cell Line THP-1

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***Listeria monocytogenes* is a facultative intracellular pathogen which enters cells by endocytosis and reaches phagolysosomes from where it escapes and multiplies in the cytosol of untreated cells. Exposure of macrophages to gamma interferon (IFN-γ) restricts *L. monocytogenes* to phagosomes and prevents its intracellular multiplication. We have tested whether IFN-γ also modulates the susceptibility of *L. monocytogenes* to antibiotics. We selected drugs from three different classes displaying marked properties concerning their cellular accumulation and subcellular distribution, namely, ampicillin (not accumulated by cells but present in cytosol), azithromycin (largely accumulated by cells but mostly restricted to lysosomes), and sparfloxacin (accumulated to a fair extent but detected only in cytosol). We used a continuous line of myelomonocytic cells (THP-1 macrophages), which display specific surface receptors for IFN-γ, and examined the activity of these antibiotics against *L. monocytogenes* Hly^+ (virulent variant) and *L. monocytogenes* Hly^- (a nonvirulent variant defective in hemolysin production). Untreated THP-1 and phorbol myristate acetate-differentiated THP-1 were permissive for infection and multiplication of intracellular *L. monocytogenes* Hly^+ (virulent variant). All three antibiotics tested were bactericidal against this *Listeria* strain when added to an extracellular concentration of 10× their MIC. After preexposure of THP-1 to IFN-γ, *L. monocytogenes* Hly^+ was still phagocytosed but no longer grew intracellularly. The activity of ampicillin became almost undetectable (antagonistic effect), and that of azithromycin was unchanged (additive effect with that of IFN-γ), whereas that of sparfloxacin was markedly enhanced (synergy). A similar behavior (lack of bacterial growth, associated with a loss of activity of ampicillin, an enhanced activity of sparfloxacin, and unchanged activity of azithromycin) was observed in cells infected with *L. monocytogenes* Hly^-. This modulation of antibiotic activity, which we ascribe to the change of subcellular localization of *L. monocytogenes* caused by IFN-γ or by the lack of virulence factor, could result from a change in bacterial responsiveness to antibiotics, a modification of the drug activity, or differences in drug bioavailabilities between cytosol and phagosomes.**

Listeria monocytogenes is a tissue-invasive, gram-positive bacterium responsible for severe infections in humans and a wide variety of animal species (9). *L. monocytogenes* is actively internalized by host cells and incorporated into phagolysosomes. One likely determinant of its pathogenicity is the elaboration of a sulfhydryl-activated hemolysin (Hly) which allows the phagocytosed *L. monocytogenes* to egress from phagolysosomes and to be released free within the cytoplasm (18, 21, 28), thereby escaping many of the cell defense mechanisms. The nonhemolytic (Hly^-) variant, created by transposon Tn*545* mutagenesis (9), remains confined in the phagosomal compartment (11), is unable to multiply therein, and, therefore, is nonpathogenic (10). Exposure of macrophages to gamma interferon (IFN-γ) prior to internalization of *L. monocytogenes* also prevents *L. monocytogenes* from reaching the cytoplasm, making the bacteria stay in phagosomes (22).

We and others have suggested that the activity of an antibiotic against an intracellular bacterium is determined, beyond its intrinsic antimicrobial properties, by key cellular pharmacokinetic and pharmacodynamic parameters which include not only its level of cellular accumulation but, most importantly, its bioavailability (i.e., its ability to have access to the site of infection) as well as its capacity to effectively express sufficient activity at this site, taking into account the physicochemical conditions prevailing therein and the metabolic status of the target bacteria (2, 31, 32). The use of IFN-γ and of *L. monocytogenes* offers the attractive opportunity to examine the activity of antibiotics against the same organism sojourning in two distinct subcellular compartments (cytosol versus phagosomes), where they display different rates of multiplication, to assess the importance of the parameters underlined above. Earlier studies from our laboratory (see references 26 and 31 for reviews) have shown that three classes of antibiotics, namely, the β-lactams (which do not accumulate in cells but nevertheless enter them and gain access to their cytoplasm [23]), fluoroquinolones (which show a fair degree of accumulation but seem largely if not exclusively localized in the cytosol [7]), and macrolides (which may show very high levels of cellular accumulation but are largely stored in lysosomes [7, 8]), could be particularly interesting in this context. The aim of the work described here was therefore to quantitatively assess the activity of a typical member of each of these three classes of antibiotics (ampicillin, sparfloxacin, and azithromycin) on intracellular *L. monocytogenes* Hly^+ and Hly^- in control macrophages and in macrophages exposed to IFN-γ. Since the activity of IFN-γ is largely species specific (14), the study was

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performed with a continuous cell line of human macrophages (THP-1) and human recombinant IFN-γ.

MATERIALS AND METHODS

Cells. THP-1 cells, a myelomonocytic cell line derived from the blood of a 1-year-old boy with acute monocytic leukemia (29), were propagated in RPMI 1640 medium supplemented with 10% decomplemented (56°C, 30 min) fetal calf serum and 2 mM glutamine, in an atmosphere of 95% air–5% CO_2 at 37°C. Cells were subcultured every third day at an initial density of 2×10^5 cells per ml.

Cell differentiation and activation. THP-1 cells (10^6 cells per ml) in suspension were differentiated by a first incubation with phorbol myristate acetate (PMA; 0.16 μM; Sigma Chemical Co., St. Louis, Mo.) for 24 h at 37°C in plastic petri dishes (Nunclon; Nunc Inc., Naperville, Ill.). Cells became adherent and were further activated by a subsequent exposure to recombinant human IFN-γ (100 U/ml; specific activity, 2×10^7 U/mg of protein; Boehringer, Mannheim, Germany) for 24 h at 37°C. In some experiments, cells were activated without previous differentiation, in which case they did not adhere to plastic and were kept as a loose suspension in the dishes.

Bacterial strains and cultures. *L. monocytogenes* Hly^+ and Hly^- were obtained from P. Berche (Laboratoire de Microbiologie, Faculté de Médecine Necker, Paris, France). The wild strain (Hly^+) is a collection type strain, EGD, from the Trudeau Institute (1982). Its nonhemolytic, nonvirulent variant (Hly^-) was obtained by insertion of the transposon Tn*545* within the hemolysin structural gene of the wild strain (10, 11). Hemolysin production in both strains was controlled by growth on 5% horse blood tryptic soy agar (Becton Dickinson). For use in cell culture experiments, bacteria were grown in tryptic soy broth, harvested in log-phase growth (≈10^8 bacteria per ml), and stored in 1-ml aliquots at −80°C until required. For each experiment, a sample of the frozen stock was rapidly thawed and inoculated in 50 ml of tryptic soy broth. After 18 h of incubation at 37°C, bacteria were washed once in phosphate-buffered saline (PBS) and used after appropriate dilution in RPMI 1640 medium supplemented with 10% decomplemented fetal calf serum. The number of viable bacteria was determined by plating 0.1-ml aliquots of serial dilutions on tryptic soy agar. CFU were counted after a 24-h incubation at 37°C.

Determination of MICs. To compare the intracellular activities of antibiotics at a precise equipotent extracellular concentration, MICs were determined in the culture medium of the macrophages (RPMI 1640–10% decomplemented fetal calf serum) by arithmetic dilution (0.1-μg increments) and at a constant inoculum (10^6 bacteria per ml). The MIC was defined as the lowest concentration of each antibiotic giving no visible bacterial growth (naked-eye examination) after an 18-h incubation at 37°C. MICs measured in these conditions were 0.2 μg/ml for ampicillin, 0.4 μg/ml for azithromycin, 1.2 μg/ml for sparfloxacin, and 0.8 μg/ml for gentamicin against *L. monocytogenes* Hly^+ and 0.3 μg/ml for ampicillin, 0.6 μg/ml for azithromycin, 2.5 μg/ml for sparfloxacin, and 0.8 μg/ml for gentamicin against *L. monocytogenes* Hly^-. These values were within ±1 geometric dilution from those found in broth by the conventional technique in preliminary experiments (data not shown).

Infection of THP-1 macrophages and assessment of antibacterial activity of antibiotics. All experiments were conducted in multiwell dishes (4-cm-diameter wells; 2 ml of medium) at an initial density of approximately 5×10^5 cells per ml. For adhering cells, the culture medium was removed by aspiration and replaced with fresh medium containing the bacteria (5×10^6 CFU per well for *L. monocytogenes* Hly^+ and 2×10^7 CFU per well for *L. monocytogenes* Hly^-). The cells were then incubated at 37°C for 1 h, the medium was decanted, and the cell sheet was washed with prewarmed PBS (six times with 4 ml). At this time, the ratio of viable bacteria (CFU counting) to macrophages was approximately 1:1. Cells were then reincubated with a control medium or with a medium containing the antibiotics (at an extracellular concentration of 10 times their MIC) for up to 5 h. At selected time intervals, this medium was aspirated and the cell sheet was washed thoroughly with ice-cold PBS. Cells were collected by gentle scraping and lysed in distilled water. In this process, the cell sample was diluted at least 2,000-fold on a volume basis, so that carried-over antibiotic could not interfere with the CFU determination. The resulting suspension was used for determination of the number of viable bacteria by colony counting after plating on tryptic soy agar (CFU) and for assay of total cell protein (15). For nonadhering cells, a similar procedure was used, except that every step of medium removal and cell washing was made by bringing the cells in suspension by gentle shaking and separating them from the suspension medium by centrifugation at 1,000 rpm for 10 min in a Damon/IEC CRU 5000 centrifuge. All results are expressed as CFU per milligram of cell protein (as an average, 1 mg of cell protein corresponds to approximately 10^6 THP-1 cells).

Statistical analysis. Differences between two groups were tested for significance by Student's *t* test with the Excell software (Microsoft Corporation, Bellevue, Wash.).

Antibiotics. Antibiotics were obtained as powder suitable for in vitro susceptibility testing from suppliers as follows: gentamicin (Geomycin; commercial mixture of the three main components C_1, C_{1a}, and C_2) from Schering Plough Belgium, Brussels, Belgium; ampicillin from Sigma Chemical Co.; azithromycin from Pfizer, Brussels, Belgium; and sparfloxacin from Rhône-DPC Europe, Paris, France.

RESULTS

In a first series of experiments, cells were infected with the virulent strain of *L. monocytogenes* (Hly^+) and then exposed for up to 5 h to antibiotics. Figure 1 shows the number of viable bacteria recovered at various times during this period from control cells (A), PMA-differentiated cells (B), cells activated by IFN-γ (C), and cells first differentiated with PMA and then further exposed to IFN-γ (D). In control cells, bacteria grew almost 10-fold in the absence of antibiotic. A similar pattern was seen if macrophages were incubated in the presence of gentamicin at an extracellular concentration of 8 μg/ml (i.e., 10× its MIC in the extracellular milieu), demonstrating a total lack of effect of this antibiotic (data not shown). In contrast, all three other antibiotics were active, yielding rather closely similar bactericidal effects, with a weak but nevertheless distinctly perceivable advantage for sparfloxacin. In PMA-differentiated cells, bacteria grew more slowly compared with control cells in the absence of antibiotics. Ampicillin and azithromycin showed essentially the same effect as in control cells, but sparfloxacin was almost two times more bactericidal. This increase, however, was not statistically significant. Cells activated with IFN-γ could still be infected but became nonpermissive for growth of *L. monocytogenes* Hly^+. They actually demonstrated a slightly bactericidal activity in the absence of antibiotic (gentamicin was inactive, demonstrating that the bacteria had been effectively internalized). Compared with PMA-treated cells, the activity of sparfloxacin in IFN-γ-treated cells was still further increased. It reproducibly proceeded in a biphasic fashion, causing first an approximately 30-fold decrease of the inoculum over the first 1 to 2 h, followed by a plateau, and yielding a further 30-fold decrease after 5 h (this biphasic pattern of the time-kill curve of sparfloxacin was consistently seen in all IFN-γ-activated cells; see below). In contrast, ampicillin showed almost no net antibiotic activity, i.e., it did not significantly influence the slight decrease of CFU already seen in IFN-γ-treated cells. Azithromycin overall activity appeared increased compared with control cells, but in contrast to sparfloxacin, this effect could entirely be accounted for by the action of IFN-γ itself. All these changes in bacterial growth and antibiotic response were not due to cell differentiation, since there was essentially no difference in the responses observed between cells exposed to both PMA and IFN-γ (in succession) and cells exposed to IFN-γ only (Fig. 1C versus D).

IFN-γ is known to confine the virulent form of *L. monocytogenes* (Hly^+) in phagosomes (22) but exerts also many other effects on macrophages which could have contributed to the results described above. To help to ascribe the effects seen to the change in the subcellular localization of the bacteria, experiments were repeated with control cells infected with *L. monocytogenes* Hly^-, a variant which is defective for intracytosolic access (21). Figure 2A shows that untreated THP-1 macrophages handled *L. monocytogenes* Hly^- almost exactly like IFN-γ-treated cells for the virulent Hly^+ variant. Thus, in both cases, the inoculum slightly decreased over the observation period but with no significant difference between the two sets of data. This, therefore, demonstrates that treatment with IFN-γ and lack of virulence factor are not additive concerning intracellular bacterial killing and could therefore act at some point through the same mechanism. Because of this, all subsequent experiments with *L. monocytogenes* Hly^- were performed with IFN-γ-pretreated cells. Figure 2B shows that the addition of antibiotics yielded almost exactly the same effects on the Hly^- strain as those observed above for the Hly^+ strain in IFN-γ-activated cells. Thus, ampicillin had only a minimal activity, sparfloxacin exerted a marked bactericidal effect with

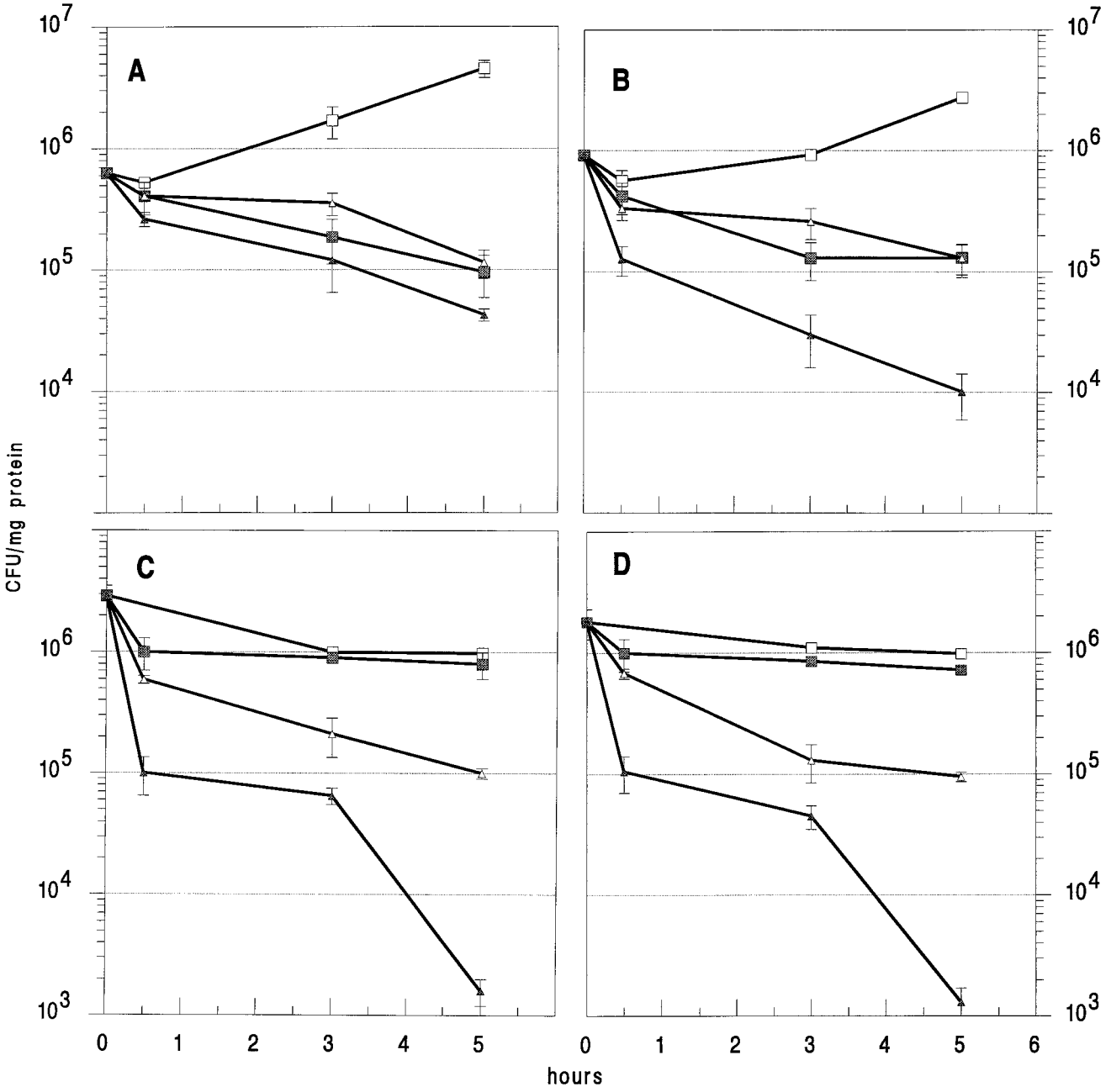

FIG. 1. Variation of the number of viable *L. monocytogenes* Hly^+ (CFU) in THP-1 macrophages during a 5-h incubation after phagocytosis in the absence (open square) or in the presence of antibiotics (shaded square, ampicillin; open triangle, azithromycin; shaded triangle, sparfloxacin; extracellular concentration, 10× MIC). (A) No pretreatment (cells were grown in suspension); (B) THP-1 macrophages differentiated by preexposure to PMA (0.16 μM; 24 h; adhering cells); (C) THP-1 macrophages activated by IFN-γ (100 μg/ml; 24 h; cells were maintained in suspension); (D) THP-1 macrophages exposed first to PMA and then further activated by IFN-γ (adhering cells). Values are given as arithmetic means of the actual CFU per milligram of macrophage protein (corresponding to an average of 10^6 cells) ± standard deviation ($n = 3$).

a biphasic pattern, and azithromycin showed an intermediate behavior. Gentamicin at 10× its MIC (8 μg/ml) was without effect in all these situations (data not shown).

DISCUSSION

The present study examines in a simple in vitro experimental system the growth patterns of *L. monocytogenes* in macrophages, when these cells have been pretreated with IFN-γ and/or are exposed to antibiotics with distinct cellular pharmacokinetic properties. THP-1 cells share many of the characteristics of human macrophages including the expression of receptors for cytokines (25) and IFN-γ in particular (confirmed in our laboratory; data not shown). Yet, they are unable to kill intracellular bacteria when unstimulated and even provide a favorable environment for the growth of many virulent strains (viz., this study and unpublished data). This not only tends to maximize the antibacterial potential of antibiotics and other

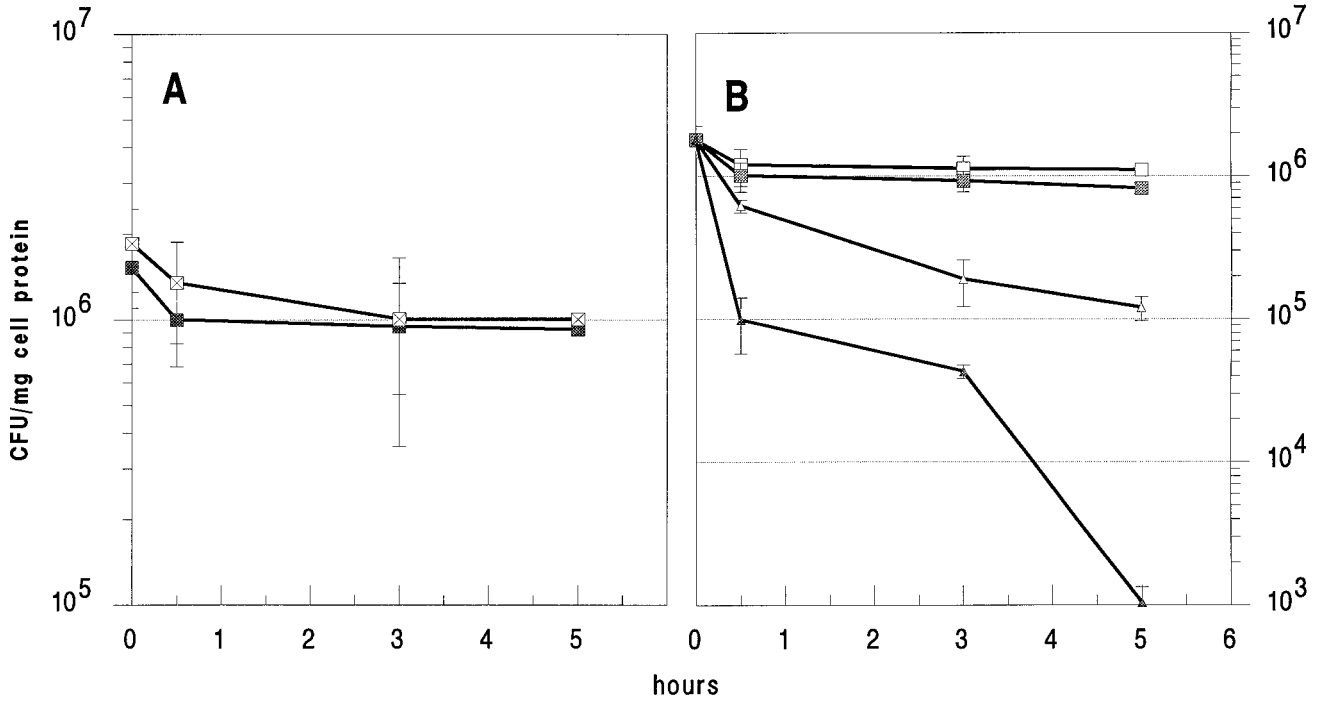

FIG. 2. Variation of the number of viable *L. monocytogenes* Hly$^-$ cells in control THP-1 macrophages during a 5-h period after phagocytosis. Cells are maintained in suspension. (A) Influence of IFN-γ; open square, control cells; shaded square, cells pretreated for 24 h with IFN-γ at 100 U/ml. (B) Cells were pretreated with IFN-γ (100 U/ml; 24 h), allowed to phagocytose bacteria, and thereafter reincubated in the absence (open square) or in the presence of antibiotics (shaded square, ampicillin; open triangle, azithromycin; shaded triangle, sparfloxacin; extracellular concentrations, 10× MIC). Data were obtained and are presented as for Fig. 1.

antibacterial agents but also allows measurement independent of the main host defenses. The experimental setup used is therefore mainly aimed at pharmacological studies. It is also strictly geared towards the evaluation of drugs against intracellular bacteria. These simplifications and departures from the situation developing in vivo during *L. monocytogenes* infection (in which bacteria infect both intracellular and extracellular compartments, effective host defense mechanisms are often operating, and both actively thriving and dormant bacteria are encountered) are meant to help in unraveling and clearly delineating the basic pharmacodynamic properties of interferon and antibiotics within the context of intracellular infection.

The data presented in this paper show first that THP-1 macrophages are indeed a suitable host for invasion and intracellular multiplication of a virulent strain of *L. monocytogenes*. Significant contribution of extracellular organisms to the bacterial growth seen in control cells can reasonably be ruled out since gentamicin exerted no effect even though both strains used were highly sensitive to this antibiotic when tested in broth or in the culture medium. The data show next that THP-1 cells become nonpermissive for intracellular growth when pretreated with human recombinant IFN-γ. Most interestingly, the virulent Hly$^+$ strain is actually handled by interferon-activated cells essentially as the nonvirulent Hly$^-$ variant is by control cells. The mode of action of interferon against intracellular *L. monocytogenes*, and especially the potential role of reactive nitrogen oxidant species in human macrophages, is not established (see references 3, 12, 19, 20, and 24 for conflicting reports). The similarity of behavior between the virulent (Hly$^+$) strain in interferon-treated cells and the nonvirulent (Hly$^-$) strain in control cells, however, makes unlikely a direct effect of interferon on the virulent bacteria, or an effect mediated by nonspecific antibacterial species in the macrophages. The data actually are more in agreement with the suggestion of Portnoy et al. (22) that interferon acts by blocking the action of hemolysin in the virulent strain or by making the phagosomal membrane resistant to its lytic action. The slight killing of the Hly$^-$ bacteria observed in control cells, as well as that of the Hly$^+$ strain in cells activated with interferon, probably results from the unfavorable environment of the phagosomes for *L. monocytogenes*.

A third critical observation is that ampicillin is almost as active as azithromycin or sparfloxacin against *L. monocytogenes* Hly$^+$ in control and PMA-differentiated THP-1 macrophages. Many authors have claimed that β-lactams do not penetrate eukaryotic cells (1, 4, 17, 32), which the present experiment would tend to disprove. The lack of effect of gentamicin, which, over the short period of time of our experiments, is unlikely to have penetrated cells to a significant extent (30), rules out that the effect of ampicillin is on extracellular bacteria, or on bacteria that are in a cell compartment easily accessible to totally hydrophilic molecules. Quantitative measurements of ampicillin cellular accumulation and localization in uninfected J774 macrophages (a murine cell line) have actually revealed that this antibiotic reaches, in less than 30 min, a cytosolic concentration approximately equal to or only slightly lower than the extracellular concentration when the experiments are conducted under conditions of culture similar to what was used here (26). We have not measured the concentration of ampicillin in *L. monocytogenes*-infected THP-1, but we may reasonably anticipate a similar behavior. Because the extracellular concentration of ampicillin was set in the present experiments at 10× its MIC, its cytosolic concentration may therefore be expected to reach a value at which the antibiotic may become bactericidal, which may account for the results observed. Quite

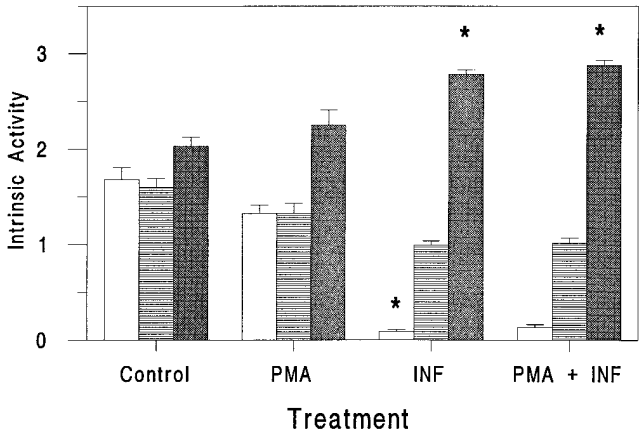

FIG. 3. Influence of the exposure of THP-1 macrophages to PMA, to IFN-γ, and to these two agents in succession on the intrinsic antibacterial activity of ampicillin (open bar), azithromycin (striped bar), or sparfloxacin (shaded bar) against phagocytosed *L. monocytogenes* Hly^+. Intrinsic activity is defined as the bactericidal activity due to the antibiotic itself, disregarding the contribution of PMA and/or IFN-γ, in reducing the number of bacteria. Values shown have been calculated from the 5-h data presented in Fig. 1A to D and are the $\log_{10}$ of the ratio between the CFU collected from cells incubated without antibiotic but subjected to the pretreatments indicated and the CFU collected from cells incubated in the presence of each of the drugs studied after the different pretreatments. Values are the arithmetic means of these ratios (± standard error of the mean; $n = 3$). Values differing from those of untreated cells by the Student t test ($P < 0.05$) are shown with an asterisk.

surprisingly, the data also show that the activities of azithromycin and sparfloxacin in control cells are not considerably higher than that of ampicillin, although these drugs are commonly reported to accumulate in large amounts in cells (30) (data from J774 macrophages would predict that, within 5 h, sparfloxacin and azithromycin should accumulate approximately 10- and 30-fold, respectively [6, 7, 26]). Our observations, therefore, would imply that cell-associated sparfloxacin and azithromycin are considerably less bioavailable than ampicillin towards cytosolic *L. monocytogenes* in infected cells (difference between the subcellular localization of the bacteria and the bulk of the drug; partial binding of the drug to cell constituents?) or that these drugs are partly prevented from exerting their action in the cytosolic milieu (impairment of drug activity; partial bacterial unresponsiveness?).

A fourth salient observation is that IFN-γ strikingly modulates the activities of the three antibiotics studied. Figure 3 shows the intrinsic antibacterial activities (i.e., the change of bacterial counts in antibiotic-exposed cells compared with that seen in cells unexposed to the drug) observed in untreated cells and in cells treated with PMA and/or interferon. It clearly appears that interferon exerts a complete antagonism towards ampicillin, whereas it acts synergistically with sparfloxacin; indifference, and therefore an additive effect only, is seen with azithromycin. The same effects (loss of activity of ampicillin, increased activity of sparfloxacin, and no change in the net activity of azithromycin) are seen when using the Hly^- variant in comparison with the Hly^+ strain. We therefore suggest that this modulation in antibiotic activity is primarily due to the change in the subcellular localization of the target bacteria from cytosol to phagosomes or to its direct consequences on cell metabolism. Thus, the data could be interpreted as resulting from a modification of the bioavailability of the antibiotics, a change in the expression of the activity of the drugs (because of the different environment in which they need to act [phagosomes versus cytosol], or a modification of the bacterial responsiveness (in relation to the lack of multiplication of the bacteria in cells pretreated with interferon).

Within the context of the first interpretation, we reported that about one-third of cell-associated pefloxacin and ciprofloxacin moves from cytosol to phagosomes of J774 macrophages when these cells are infected with *Legionella pneumophila* (7, 27), i.e., where the bacteria sojourn (13). This implies that fluoroquinolones should become highly concentrated therein, because the volume of phagosomes is considerably smaller than that of the cytoplasm. If this phenomenon also occurs in THP-1 infected with *L. monocytogenes*, it could account for the increased activity of sparfloxacin when the bacteria are constrained to phagosomes. Drug subcellular redistribution and concentration in infected phagosomes would therefore appear as a general property of fluoroquinolones. Cell fractionation and dose-dependence kill studies are, however, needed to further prove this hypothesis. Within the same context, it is tempting to speculate that ampicillin fails to show activity against phagosomal listeriae (and most other phagosomal organisms as well, unless large extracellular concentrations are used [26, 31]) because it is largely excluded from phagosomes which display a slightly acidic pH (see discussion in reference 23).

Another explanation, however, could be that listeriae which cannot escape from phagosomes become tolerant of ampicillin merely because they stop multiplying. It is indeed well known that β-lactams are dependent upon bacterial division to exert their bactericidal effects (16). If such is the case, however, we also need to hypothesize that fluoroquinolones are more active against nonmultiplying than against actively multiplying listeriae and/or are considerably more active in phagosomes than in cytosol. Another possibility, however, is that interferon modulates the accumulation of antibiotics, towards a decrease for ampicillin and an increase for sparfloxacin. Here again, kill-kinetic studies, in an environment that mimics the phagosomal milieu, associated with drug accumulation and localization studies, would be of large interest. These studies may also help to explain the biphasic character of the killing of listeriae in IFN-γ-treated cells.

Endogenous IFN-γ is an important determinant for resolution of *L. monocytogenes* infection in vivo (5), by promoting the clearance of these bacteria by activated macrophages. Our data indicate that β-lactams and interferon become antagonistic against intracellular bacteria in interferon-treated cells. Assuming that this conclusion also holds true in vivo, it would mean that the action of ampicillin will be limited to extracellular bacteria or to bacteria growing in cells insensitive to the action of interferon. Ampicillin would therefore be largely ineffective by itself in eradicating the infection. Conversely, fluoroquinolones may appear as drugs of choice for this purpose since they effectively cooperate with interferon to achieve fast clearance of intraphagocytic listeriae. These issues may be well worth critically examining in appropriately designed animal and/or clinical trials.

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REFERENCES

1. **Alexander, J. W., and R. A. Good.** 1968. Effect of antibiotics on the bactericidal activity of human leukocytes. J. Lab. Clin. Med. **71:**971–983.
2. **Baillie, A. J.** 1984. Intracellular infection and drug targeting. Pharm. Int. **5:**168–172.
3. **Bermudez, L. E.** 1993. Differential mechanisms of intracellular killing of Mycobacterium avium and Listeria monocytogenes by activated human and murine macrophages. The role of nitric oxide. Clin. Exp. Immunol. **91:**277–281.
4. **Brown, K. N., and A. Percival.** 1978. Penetration of antimicrobials into tissue culture cells and leucocytes. Scand. J. Infect. Dis. **14**(Suppl.)**:**251–260.
5. **Buchmeier, N. A., and R. D. Schreiber.** 1985. Requirements of endogenous interferon-gamma production for resolution of Listeria monocytogenes infection. Proc. Natl. Acad. Sci. USA **82:**7404–7408.
6. **Carlier, M. B., I. Garcia-Luque, J.-P. Montenez, P. M. Tulkens, and J. Piret.** 1994. Accumulation, release and subcellular localization of azithromycin in phagocytic and non-phagocytic cells. Int. J. Tissue React. **16:**211–220.
7. **Carlier, M. B., B. Scorneaux, A. Zenebergh, J. F. Desnottes, and P. M. Tulkens.** 1990. Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. J. Antimicrob. Chemother. **26(B):** 27–39.
8. **Carlier, M. B., A. Zenebergh, and P. M. Tulkens.** 1987. Cellular uptake and subcellular distribution of roxithromycin and erythromycin in phagocytic cells. J. Antimicrob. Chemother. **20(B):**47–56.
9. **Farber, J. M., and P. I. Peterkin.** 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. **55:**476–511.
10. **Gaillard, J. L., P. Berche, J. Mounier, S. Richard, and P. Sansonetti.** 1987. In vitro model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. Infect. Immun. **55:**2822–2829.
11. **Gaillard, J. L., P. Berche, and P. Sansonetti.** 1986. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. Infect. Immun. **52:**50–55.
12. **Granger, D. L., J. B. Hibbs, J. R. Perfect, and D. T. Durack.** 1988. Specific amino acid (L-arginine) requirement for the microbiostatic activity of murine macrophages. J. Clin. Invest. **81:**1129–1134.
13. **Horwitz, M. A.** 1983. The legionnaire's disease bacterium (*Legionella pneumophila*) inhibits phagosome-liposome fusion in human monocytes. J. Exp. Med. **158:**2108–2126.
14. **Littman, S. J., C. R. Faltyneck, and C. Baglioni.** 1985. Binding of human recombinant ^{125}I-interferon-gamma to receptors on human cells. J. Biol. Chem. **260:**1191–1195.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
16. **Maidhof, H., L. Johannsen, H. Labischinski, and P. Giesbrecht.** 1989. Onset of penicillin-induced bacteriolysis in staphylococci is cell cycle dependent. J. Bacteriol. **171:**2252–2257.
17. **Mandell, G. L.** 1972. Killing of intraleukocytic Staphylococcus aureus by rifampin: in vitro and in vivo studies. J. Infect. Dis. **125:**486–490.
18. **Mounier, J., A. Ryter, M. Rondon, and P. J. Sansonetti.** 1990. Intracellular and cell-to-cell spread of L. monocytogenes involves interaction with F-actin in the enterocyte-like cell line Caco-2. Infect. Immun. **58:**1048–1058.
19. **Murray, H. W., Y. Rubin, S. M. Carriero, M. Harris, and E. A. Jaffe.** 1985. Human mononuclear phagocyte antiprotozoal mechanisms: oxygen-dependent vs oxygen independent activity against intracellular Toxoplasma gondii. J. Immunol. **134:**1982–1987.
20. **Murray, H. W., and R. F. Teitelbaum.** 1992. L-Arginine dependent reactive nitrogen intermediates and the antimicrobial effect of activated human mononuclear phagocytes. J. Infect. Dis. **165:**513–517.
21. **Portnoy, D. A., P. S. Jacks, and D. J. Hinrichs.** 1988. Role of hemolysin for the intracellular growth of Listeria monocytogenes. J. Exp. Med. **167:**1459–1471.
22. **Portnoy, D. A., R. D. Schreibner, P. Connelly, and L. G. Tiney.** 1989. Gamma interferon limits access of Listeria monocytogenes to the macrophage cytoplasm. J. Exp. Med. **170:**2141–2146.
23. **Renard, C., H. G. Vanderhaeghe, P. J. Claes, A. Zenebergh, and P. M. Tulkens.** 1987. Influence of conversion of penicillin G into a basic derivative on its accumulation and subcellular localization in cultured macrophages. Antimicrob. Agents Chemother. **31:**410–416.
24. **Rothermel, C. D., B. Y. Rubin, and H. W. Murray.** 1986. Oxygen-independent inhibition of intracellular Chlamydia psittaci growth by human monocytes and interferon-gamma activated macrophages. J. Immunol. **137:**689–696.
25. **Sanceau, J., G. Merlin, and J. Wietzerbin.** 1992. Tumor necrosis factor-alpha and IL6 regulate IFN-gamma receptor gene expression in human monocytic THP-1 cells by transcriptional and post-transcriptional mechanisms. J. Immunol. **149:**1671–1675.
26. **Scorneaux, B.** 1993. Conception de modèles quantitatifs d'infection intracellulaire pour l'étude de l'action des antibiotiques. Ph.D. thesis. Université Catholique de Louvain, Brussels.
27. **Scorneaux, B., and P. M. Tulkens.** 1994. Intracellular infection by *L. pneumophila* and *S. aureus* modulates the subcellular distribution of fluoroquinolones but not that of macrolides in a model of J774 macrophages, abstr. E31. *In* Program and Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
28. **Tilney, L. G., and D. A. Portnoy.** 1989. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, Listeria monocytogenes. J. Cell Biol. **109:**1597–1608.
29. **Tsuchiya, S., M. Yamabe, Y. Yamaguchi, Y. Kobamyashi, T. Konno, and K. Tada.** 1980. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int. J. Cancer **26:**171–176.
30. **Tulkens, P. M.** 1991. Intracellular distribution and activity of antibiotics. Eur. J. Clin. Microbiol. Infect. Dis. **10:**100–106.
31. **Tulkens, P., and A. Trouet.** 1978. The uptake and intracellular accumulation of aminoglycosides antibiotics in lysosomes of cultured fibroblasts. Biochem. Pharmacol. **27:**415–424.
32. **van den Broek, P. J.** 1989. Antimicrobial drugs, microorganisms and phagocytes. Rev. Infect. Dis. **11:**213–245.