Synthesis of DNA, rRNA, and Protein by Rickettsia prowazekii Growing in Untreated or Gamma Interferon-Treated Mouse L929 Cells

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The syntheses of DNA, rRNA, and protein by Rickettsia prowazekii growing in mouse fibroblastic L929 cells were measured at various times after the addition of gamma interferon (IFN-γ) to correlate the inhibition of a site of macromolecular synthesis with the established IFN-γ-induced inhibition of rickettsial growth. A method was developed to measure the syntheses of DNA, rRNA, and protein by R. prowazekii during a 2-h pulse-labeling period while the rickettsiae were growing within cultured host cells that had intact macromolecular synthesis. This method involved incubation of the rickettsia-infected cells with a radioactive precursor (H,23PO4 or Tran35S-label), purification of the rickettsiae, purification of rickettsial nucleic acids, and analysis of rickettsial nucleic acids and proteins by electrophoresis and autoradiography. A key feature of the method involved the use of calculated specific activities from a densitometric analysis of gels and autoradiograms, a procedure that made the data independent of rickettsial recovery. Rickettsial DNA and rRNA syntheses were both inhibited 12 h after the addition of IFN-γ to infected cultures, whereas the synthesis of rickettsial proteins was not inhibited at this time. In contrast, at 20 h after the addition of IFN-γ, rickettsial DNA, rRNA, and protein syntheses were all inhibited.

Rickettsia prowazekii, the etiological agent of epidemic typhus, is an obligate intracellular bacterium that grows directly in the cytoplasm of its host cells, unbound by a phagosomal or phagolysosomal membrane (32, 35). Gamma interferon (IFN-γ) induces cultured fibroblasts to express antimicrobial activity against R. prowazekii (24, 28, 37, 38), and this lymphokine is important in host defense against Rickettsia conorii infection in mice (12). However, the mechanisms of action of IFN-γ against rickettsiae in vitro and in vivo remain to be established. Since murine IFN-γ does not induce antirickettsial activity in human cells and the antirickettsial effect of IFN-γ is partially alleviated by cycloheximide (24, 37), it is almost certain that IFN-γ does not affect the rickettsiae directly. IFN-γ interacts with specific plasma membrane receptors and causes a series of cytoplasmic and nuclear events that lead to the appearance of IFN-γ-induced gene products (2, 13, 15, 17, 18, 23, 33). The IFN-γ-induced product(s) that is responsible for the inhibition of rickettsial growth may act on the rickettsiae (directly or indirectly) to inhibit rickettsial growth or may alter the host cell so that sufficient quantities of the metabolites needed by R. prowazekii for growth are not available. It is also possible that IFN-γ-induced gene products inhibit rickettsial growth both by their direct actions on rickettsiae and by nutritional deprivation. Amino acid deprivation after IFN-γ treatment of fibroblasts, especially tryptophan deprivation (which is responsible for human IFN-γ-induced inhibition of the growth of Toxoplasma gondii and Chlamydia psittaci [5, 14]), does not occur in the establishment of antirickettsial activity in mouse L929 cells and cannot be the sole factor involved in the antirickettsial activity induced by human IFN-γ (27). Previous studies, such as the aforementioned one, focused on changes in the host cell wrought by IFN-γ treatment rather than changes in the metabolism of the parasite. It is clear that, regardless of the mechanism of inhibition of rickettsial growth by IFN-γ, rickettsial synthesis of macromolecules (DNA, rRNA, and protein) should be inhibited sometime after the treatment of rickettsia-infected cultures with IFN-γ. The present study initiates an investigation of rickettsial metabolism as affected by IFN-γ treatment of the host cell.

The previously described methods used to measure the synthesis of macromolecules by rickettsiae growing within the cytoplasm of their host cells (i.e., in situ) made use of inhibitors to suppress the synthesis of eucaryotic macromolecules so that rickettsia-specific signals could be obtained (see, for example, references 1 and 21). In addition, in such studies, the rickettsiae were labeled for as many generations as possible to increase the magnitude of the rickettsial signals. However, for study of the effect of IFN-γ on the rates of synthesis of rickettsial macromolecules in situ, a pulse of label that does not last for a rickettsial generation must be used, and eucaryotic macromolecular synthesis inhibitors cannot be used because the antirickettsial action of IFN-γ is probably dependent on de novo mRNA and protein syntheses of the host cell. Therefore, the elimination of labeled host cell macromolecules is an essential part of an isotopic method for measuring the rates of synthesis of rickettsial macromolecules. In addition, for comparison of the rates of synthesis of rickettsial macromolecules from untreated and IFN-γ-treated cultures, consideration must be given to the problem of variable recoveries of rickettsial macromolecules from sample to sample.

In this study, we report that rickettsial DNA and rRNA syntheses are both inhibited 12 h after the addition of IFN-γ to rickettsia-infected cultures, whereas the synthesis of rickettsial proteins is not inhibited at this time. In contrast, at 20 h after the addition of IFN-γ, rickettsial DNA, rRNA, and protein syntheses are all inhibited.

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MATERIALS AND METHODS

Cell cultures. Mouse L929 cells were cultured in Eagle minimal essential medium (MEM) supplemented with 10% heat-treated (56°C for 30 min) newborn bovine serum (NBS). The cells were grown as monolayers in a humidified atmosphere of 3% CO2 in air at 34°C. During the incubation of infected cells with H32PO4 or Tran35S-label for 2 h, MEM was phosphate free, had a reduced concentration of L-methionine (0.4 mM), and was supplemented with 10% NBS (deficient MEM-NBS).

Rickettsiae. R. prowazekii Madrid E (yolk sac passage 281) was inoculated into 6-day-old embryonated antibiotic-free hen eggs (Truslow Farms, Chestertown, Md.). The rickettsiae were harvested and purified from the infected yolk sacs 8 days later as previously described (26). Samples of rickettsiae suspended in sucrose-phosphate-glutamate (SPG)-magnesium (SPG-Mg) solution (0.218 M sucrose, 3.76 mM KH2PO4, 7.1 mM K2HPO4, 4.9 mM potassium glutamate, 10 mM MgCl2 [pH 7.0]) (3) were stored at −80°C. The numbers of viable rickettsiae were estimated on the basis of their hemolytic activity (31).

IFN-γ and other reagents. Recombinant murine IFN-γ derived from Escherichia coli (1.9 × 107 U/mg) was generously provided by Genentech, Inc. (South San Francisco, Calif.). The IFN-γ was assayed to determine antiviral units as previously described (25, 30). Guanidinium thiocyanate was purchased from Fisher Chemical, Fisher Scientific, Fair Lawn, N.J., and DNase I was purchased from Sigma Chemical Co., St. Louis, Mo. H32PO4 (carrier free) and Tran35S-labeled L-methionine [1,058 Ci/mmol, 15% L-cysteine] were purchased from ICN Radiochemicals, Inc., Irvine, Calif. Renografin was purchased from E. R. Squibb & Sons, Princeton, N.J.

Measurement of the syntheses of DNA, rRNA, and protein by R. prowazekii in untreated and IFN-γ-treated L929 cells. (i) Overview of the method. The method used for the measurement of the synthesis of macromolecules by rickettsiae in untreated and IFN-γ-treated L929 cells was as follows: samples of the infected cells with radioactive precursors, purification of the rickettsiae, fixation of rickettsial nucleic acids, and analysis of nucleic acids and proteins by electrophoresis. Densitometric analyses of both stained gels (negative images of the stained gels) and autoradiograms of the same gels permitted the calculation of specific activities (intensity of the band on an autoradiogram/intensity of the band on a gel or a negative image), which represent the amount of incorporation of the radioactive precursors per unit of rickettsial macromolecules. The percent inhibition of rickettsial incorporation in IFN-γ-treated cultures compared with that in control cultures was calculated with the following formula: % inhibition = [1 − (specific activity of IFN-γ-treated sample/specific activity of untreated sample)] × 100.

(ii) Infection of L929 cells with rickettsiae and quantification of rickettsial infection and growth. L929 cells were detached from their plastic substratum by incubation with a trypsin-EDTA solution (ICN Biomedicals, Inc., Costa Mesa, Calif.), washed, and suspended at a concentration of 105 viable (trypan blue-excluding) cells per ml in Hanks balanced salt solution supplemented with 5 mM L-glutamic acid (monopotassium salt) and 0.1% gelatin. A portion of this suspension was infected with rickettsiae at a multiplicity of infection of approximately 20 rickettsiae per cell, and another portion was mock infected (given SPG-Mg alone). After incubation at 34°C for 1 h, the cells were washed two times, and 6 × 104 cells in 10 ml of MEM supplemented with 10% NBS were planted into each tissue culture plate (diameter, 100 mm). For determination of the initial infection, samples of the washed cell suspensions were immediately centrifuged onto microscope slides, dried, fixed, and stained as previously described (25). Coverslips were placed in some of the tissue culture plates so that the rickettsial infection could be monitored at later times. Stained slides and coverslips were examined microscopically with an oil immersion objective, the number of rickettsiae present in each of 100 cells was counted, and the percentage of cells infected and the average number of rickettsiae per infected cell were determined.

(iii) Treatment of the cells with IFN-γ, incubation with radioactive precursors, and purification of rickettsiae. After incubation of the infected cells for 24 h, IFN-γ was added to some culture plates at a final concentration of 100 U/ml. After incubation for an additional 12, 16, or 20 h, the medium was removed from two untreated and two IFN-γ-treated plates, and each plate was given 4 ml of deficient MEM-NBS plus either H32PO4 (50 μCi/ml) or Tran35S-label (15 μCi/ml). After incubation with the radioactive precursor at 34°C for 2 h, the medium was removed, and each plate was washed with 10 ml of SPG-Mg. The cells were then scraped into 5 ml of SPG-Mg, frozen in liquid nitrogen, and stored at −80°C for later processing.

The frozen samples were thawed at 37°C and vortexed for 1 min to break the host cells. After centrifugation for 10 min at 250 × g, each supernatant fluid was treated with DNase I at a final concentration of 50 U/ml for 20 min at room temperature, layered onto 30 ml of 26% Renografin in SPG, and centrifuged for 45 min at 10,000 × g. The supernatant fluids were discarded, and each of the rickettsial pellets was resuspended in 1 ml of SPG and sedimented at 10,000 × g for 20 min at 4°C.

(iv) Purification and analysis of rickettsial nucleic acids. The H32PO4-labeled rickettsiae were used as a source of genomic DNA and rRNA. The rickettsial pellets were lysed in 200 μl of guanidinium thiocyanate lysis buffer (4 M guanidinium thiocyanate, 0.1 M Tris-Cl [pH 7.5], 0.5% sarcosyl, 1% mercaptoethanol [10]), and each was incubated with 200 μl of phenol-chloroform (1:1 [vol/vol]). The aqueous phase was removed, and to it were added 20 μl of 2.5 M NaCl, 40 μl of glycogen (100 μg/ml) and 780 μl of ice-cold ethanol. After precipitation overnight at −20°C, the samples were centrifuged at 10,000 × g for 20 min at 4°C, washed once with 75% ethanol, washed once with 100% ethanol, and then dried at room temperature. Dried samples were resuspended in 10 μl of TE buffer (10 mM Tris-Cl, 1 mM EDTA [pH 8.0]), and the nucleic acids were separated by electrophoresis in 1.5% agarose gels at 5 V/cm for 2.5 to 3 h with 1× TAE buffer (40 mM Tris-acetate [pH 8.0], 1 mM EDTA). The gels were stained with ethidium bromide (0.5 μg/ml) for 30 min and then were destained with distilled water for 30 min. Photographs of the gels were made with transmitted UV light. The gels were then dried and autoradiographed on XAR-5 film (Eastman Kodak Co., Rochester, N.Y.).

(v) Analysis of rickettsial proteins. Each rickettsial pellet labeled with Tran35S-label was lysed in 6 μl of loading buffer (2% sodium dodecyl sulfate [SDS], 50 mM Tris-Cl [pH 6.8], 100 mM dithiothreitol, 0.1% bromophenol blue, 10% glycerol) at 68°C for 10 min, boiled for 6 min, centrifuged at 10,000 × g for 5 min, and electrophoresed in 10 to 15% gradient SDS-polyacrylamide gels by use of the Phast Gel system (Pharmacia LKB Biotechnology, Piscataway, N.J.). After being stained with Coomassie blue, the gels were dried at room temperature overnight, wrapped in very thin plastic, and autoradiographed on Kodak XAR-5 film.
(vi) Image densitometry. The intensities of the bands on the stained protein gels, the negative images of the ethidium bromide-stained nucleic acid gels, and the autoradiograms of the gels were quantitated with a 1D/2D soft laser scanning densitometer and an ERIS reporting-integrating system (Biomed Instruments, Inc., Fullerton, Calif.).

Statistics. Data were analyzed by the t test. Percent inhibition data were used for statistical analysis.

RESULTS

Development of a method to measure the synthesis of macromolecules by R. prowazekii growing in L929 cells. Development of a method to measure the synthesis of macromolecules by rickettsiae growing within their host cells required the consideration of several factors: (i) selection of appropriate isotopic precursors on the basis of the metabolism of the host cells and the pathways used by rickettsiae for synthesizing macromolecules, (ii) strategies for increasing the amounts of radioactive precursors incorporated by the rickettsiae so that measurable signals could be obtained, (iii) elimination of host cell macromolecules during processing of the samples so that rickettsial synthesis could be specifically evaluated, and (iv) correction for losses of material that would occur during processing of the samples.

First, because R. prowazekii is not able to utilize thymidine added to infected cell cultures to synthesize its DNA (21), it is impossible to label rickettsial DNA without rickettsial RNA being labeled and impractical to label rickettsial RNA with uridine so that rickettsial DNA is not labeled. Although rickettsiae transport ribonucleotides from the host cell cytoplasm and synthesize TTP from these ribonucleotides (6, 21, 22), the expense of labeling rickettsial nucleic acids in the presence of an enormous sink of host cell biosynthetic activity contraindicated labeling with ribonucleosides. Therefore, we decided to use a large amount of H$_3$PO$_4$ to label rickettsial nucleic acids and then separate rickettsial DNA and RNA to assess their synthesis individually. Since rickettsiae can transport amino acids and incorporate them into protein (1, 4, 7, 8, 19, 34, 36), we decided to label rickettsial proteins with Tran$^{35}$S-label, a mixture of radiolabeled methionine and cysteine. In principle, any labeled amino acid could have been used, but Tran$^{35}$S-label was the most cost-effective. The rate of uptake of trichloroacetic acid-soluble Tran$^{35}$S-label by infected host cells and the rate of incorporation of H$_3$PO$_4$ into organic phosphates in infected host cells were similar in untreated cultures and cultures treated with IFN-γ for 24 h (data not shown). For enhancement of the labeling of rickettsial nucleic acids and proteins, the labeling was conducted with host cells incubated in deficient MEM-NBS, which has reduced concentrations of NaH$_2$PO$_4$ and methionine. We determined that these reduced concentrations of NaH$_2$PO$_4$ and methionine did not limit rickettsial growth in heavily infected L929 cells during a 24-h period (data not shown). These experiments ensured the validity of the conditions for the measurement of the incorporation of radioactive precursors into rickettsiae. Inhibitors of eucaryotic protein and RNA syntheses were not used to enhance the labeling of rickettsial proteins and RNA because the antirickettsial action of interferon is dependent on de novo eucaryotic protein synthesis (24, 37).

Purification of the rickettsiae by centrifugation through 30 ml of 26% Renografin in SPG largely removed contamination from host cell protein. Purification of the rickettsiae by fractionation of Renografin gradients provided unacceptable yields, and sedimentation in a 2-ml microcentrifuge tube provided unacceptable purity (data not shown). For elimination of contamination from adherent host cell DNA that sedimentation through Renografin would not remove, the samples were treated with DNase I during rickettsial purification. On the basis of its sedimentation coefficient, rickettsial rRNA could be separated from the bulk of host cell RNA by agarose gel electrophoresis. RNase was not used to eliminate contamination from host cell rRNA, because RNase is difficult to inactivate and residual RNase might have degraded rickettsial rRNA. We chose a labeling period that was a compromise between temporal resolution and signal. Since the generation time of R. prowazekii is about 10 h, the 2-h pulse used would be comparable to a few minutes of pulse labeling with E. coli but would yield significant densities on autoradiograms after exposures of about 48 h.

A comparison of rickettsial macromolecular synthesis in different samples required a method that would not be influenced by the efficiency of recovery of rickettsial macromolecules following such extensive processing of the samples. The data obtained by simply quantitating the total amount of isotope incorporated into a given macromolecular fraction would be dependent on recovery. Therefore, density-based specific activity was determined for each macromolecule to provide a measure of incorporation of the precursor per unit of the macromolecule, a measure that was independent of the total amount of the macromolecule.

Rickettsial infection and growth in experiments in which rickettsial macromolecular synthesis was measured. IFN-γ was added to L929 cells 24 h after infection of 99% ± 1% (mean ± standard deviation) of the cells with 8.5 ± 0.6 rickettsiae per infected cell. This 24-h incubation period before the addition of IFN-γ was necessary so that the numbers of rickettsiae would be large enough to allow the detection of incorporation of the radioactive precursors by the rickettsiae. Experiments with L929 cells infected with smaller numbers of rickettsiae were not feasible because of the resulting decreased amplitude of the rickettsial signal. It is possible that the intensity of the effects of IFN-γ on rickettsial macromolecular synthesis would differ in cells infected with fewer rickettsiae and that some effects of IFN-γ treatment on the rickettsiae might be missed in cells infected with large numbers of rickettsiae. At the time of addition of IFN-γ, 100% ± 0% of the cells were infected with 69 ± 5 rickettsiae per infected cell. After an additional incubation for 12, 16, or 20 h, there were too many rickettsiae per cell to permit accurate counting. However, it was estimated (from an examination of coverslips that were removed and stained 20 h after the addition of IFN-γ) that the numbers of rickettsiae in the untreated cultures had increased approximately threefold. Concluding the experiments at this point provided a low level of inhibition of rickettsial growth by IFN-γ so that the time course for the inhibition of macromolecular synthesis could be established.

Syntheses of DNA, rRNA, and protein by rickettsiae in untreated and IFN-γ-treated L929 cells. In the representative experiments shown in Fig. 1, both the stained gels and the autoradiograms of the infected samples exhibited clearly visible bands of DNA, 16S rRNA, 23S rRNA, and protein. The mock-infected samples processed in the same way as the rickettsial DNA samples was unlikely. The identities of the DNA, 16S rRNA, and 23S rRNA bands were demonstrated on the basis of their migrations in the agarose gels and their sensitivities to RNase-free DNase or DNase-free RNase. In
FIG. 1. Separation of rickettsial macromolecules. The six lanes, from left to right, in each panel were as follows: 1, infected and untreated at 12 h; 2, infected and IFN-γ treated at 12 h; 3, infected and untreated at 20 h; 4, infected and IFN-γ treated at 20 h; 5, uninfected and untreated at 20 h; and 6, uninfected and IFN-γ treated at 20 h. The six major proteins are indicated by horizontal lines. AUTORAD, autoradiogram.

most experiments, the nucleic acids purified from infected and mock-infected samples did not exhibit visible host cell rRNA bands on the agarose gels and autoradiograms. When host cell 18S rRNA and 28S rRNA bands were visible, they were clearly separated from the rickettsial 16S rRNA and 23S rRNA. The protein samples from the infected cultures had many bands, and six major (outer membrane) protein bands that corresponded to the major bands described previously were identified (20). In contrast, the samples from the mock-infected cultures had only a few, very faint bands. This result suggested that there was very little contamination of the Renografin-purified rickettsial samples with proteins from the host cells. However, it should be noted that host cell proteins that could have adhered to the rickettsiae would not have been present in the mock-infected samples. The intensities of the six major rickettsial bands were quantitated and summed, since no bands corresponding to these six major bands were observed in the Renografin-purified mock-infected samples.

Figure 2 presents the data from four experiments of this type. Rickettsial DNA, 16S rRNA, and 23S rRNA syntheses were significantly inhibited at 12 h after the addition of IFN-γ to R. prowazekii-infected L929 cells, whereas the synthesis of proteins by the rickettsiae was not significantly inhibited at this time. At 20 h after the addition of IFN-γ, the production of DNA, rRNA, and proteins by the rickettsiae was significantly inhibited. Data from two experiments suggested that the production of DNA, rRNA, and proteins by the rickettsiae was also inhibited at 16 h after the addition of IFN-γ. Thus, the synthesis of nucleic acids by R. prowazekii was inhibited before the synthesis of rickettsial proteins was inhibited. The extent of inhibition of the synthesis of rickettsial DNA, 16S rRNA, and 23S rRNA at 20 h after the

FIG. 2. IFN-γ-induced inhibition of the synthesis of rickettsial major proteins, 23S rRNA, 16S rRNA, and DNA. Percent inhibition data from four experiments (Ex) at the indicated times (hours [H]) are presented. P values (based on a one-sample t test of the null hypothesis that the mean is equal to 0) are given when the number of experiments was four.
The addition of IFN-γ did not differ significantly from the respective levels of inhibition detected at 12 and 16 h (P > 0.2). Similarly, the extent of inhibition of rickettsial protein synthesis at 20 h after the addition of IFN-γ did not differ from that observed at 16 h (P > 0.5). In no instance was the synthesis of rickettsial DNA, rRNA, or proteins completely blocked. Figure 3 shows the primary data from which the inhibitions for experiment 1 in Fig. 2 were determined. The left bar of each pair represents the densitometry units from the stained gel (chemical amount), and the right bar represents the densitometry units from the autoradiogram (radioactivity). In Fig. 3, the data for 16S and 23S rRNAs have been combined. The number at the top of each pair of bars is the ratio of the radioactivity to the chemical amount for rickettsial DNA, rRNA, and proteins at 12, 16, and 20 h. The percent inhibition was calculated from the ratios for the control and IFN-γ-treated samples. Experiment 4 provided data very similar to those from the experiment shown. However, experiments 2 and 3 (data not shown) showed decreasing specific activity in the control samples as time progressed: the rate of synthesis of macromolecule per chemical amount of macromolecule fell from 12 to 20 h in the control cultures. Although we do not know why two of four experiments showed what can be interpreted as an increase in the generation time, perhaps this finding is not surprising in experiments in which the numbers of rickettsiae per cell are large. It is noteworthy that the inhibition of macromolecular synthesis caused by IFN-γ treatment was present in experiments in which the specific activities (rates of macromolecular synthesis) in the control cultures were either constant (experiments 1 and 4) or decreasing (experiments 2 and 3).

DISCUSSION

Until now, the rickettsial growth-inhibitory properties of interferons were most often measured with a microscope. The parameters "percentage of cells infected" and "number of rickettsiae per infected cell" were ascertained from stained cells, and a comparison of these parameters in untreated and interferon-treated, X-irradiated (nongrowing) cell cultures (or a comparison of the number of rickettsiae per culture in untreated and interferon-treated, growing cell cultures) established the inhibitory potency of the treatment (9–11, 24, 27, 30, 38). An enzyme-linked immunosorbent assay (29) and a plaque assay (9–11) have also been used to assess the ability of interferons to restrict rickettsial growth. In the present study, the number of molecules of radioactive precursors incorporated during a 2-h interval into a given mass of a given macromolecular species was determined in untreated and interferon-treated cells. One method is not superior to the others; they are complementary, with distinct and different strengths and weaknesses.

By 16 h after the addition of IFN-γ, rickettsial syntheses of protein, DNA, and rRNA were all inhibited. This inhibition was not merely due to a loss or destruction of rickettsiae, since the specific activity basis of the measurements determined the inhibition of the incorporation of precursors into the recovered rickettsial macromolecules. However, these measurements cannot distinguish between a portion of the rickettsiae being totally inhibited (with the remainder being uninhibited) and all of the rickettsiae being partially inhibited. Moreover, if the cultures had a mixture of totally uninhibited and totally inhibited rickettsiae and the recoveries of these populations differed, then the observed inhibition would be affected.

In contrast to the data obtained at 16 and 20 h, at 12 h after the addition of IFN-γ the rates of synthesis of DNA and rRNA by the rickettsiae were both inhibited by about 75%, but there was no detectable inhibition of the rate of synthesis of protein by the rickettsiae. These results did not suggest that every protein was synthesized at the control rate; rather, the conclusion was that the synthesis of the major outer membrane proteins was not significantly inhibited. In fact, it is reasonable to suggest that selective inhibition of the synthesis of some proteins occurred. Although the rate of synthesis of rickettsial mRNA could not be measured in the present study, the short half-life of rickettsial mRNA (less than 15 min) observed in other studies (5a, 34) suggests that the inhibition of rickettsial mRNA synthesis in IFN-γ-treated cells must have a time course very similar to that for the inhibition of rickettsial protein synthesis. This suggestion implies that rickettsial rRNA synthesis was inhibited earlier than rickettsial mRNA synthesis. The unlikely alternative would be that rickettsial rRNA synthesis was inhibited with the same time course as rickettsial rRNA synthesis but that mRNA degradation was inhibited.

It is not clear how IFN-γ-induced gene products cause inhibition of the synthesis of rickettsial DNA and rRNA but not protein at 12 h. It is possible that both DNA synthesis...
and rRNA synthesis are inhibited contemporaneously by either the same or a different IFN-γ-induced gene product(s). Alternatively, inhibition of the synthesis of one of these nucleic acids may shortly thereafter be followed by or cause inhibition of the synthesis of the other. Similarly, the subsequent inhibition of the synthesis of rickettsial protein (and rickettsial mRNA) may be either the effect of a different IFN-γ-induced gene product or a sequela of the earlier nucleic acid inhibition. In the former scenario, attention would be focused at the level of IFN-γ-induced gene product regulation and activity; whereas in the latter scenario, attention would be focused within the rickettsiae at the level of global regulation responses to unbalanced growth.

It is not known whether IFN-γ-induced gene products exert their inhibitory effects on rickettsial macromolecular synthesis by acting on the rickettsiae, the host cells, or both. It is important to note that the presence of inner and outer membranes in R. prowazekii should prevent the entry of IFN-γ-induced gene products themselves into the rickettsiae. Thus, many of the mechanisms involved in the antiviral and antiproliferative effects of IFN-γ in eucaryotic cells are difficult to apply to rickettsiae. However, IFN-γ-induced gene products might exert antirickettsial effects by binding to the rickettsial surface. Alternatively, some permeant toxic factor(s) made by an IFN-γ-induced gene product(s) in the host cell cytoplasm might inhibit the synthesis of rickettsial macromolecules by interfering with rickettsial metabolism or energy transduction. IFN-γ-induced gene products might inhibit in R. prowazekii the systems of transport of essential nutrients that the rickettsiae obtain from the host cell cytoplasm. Another possibility is that IFN-γ-induced gene products modify the host cell cytoplasm so that sufficient quantities of essential nutrients are not available for the rickettsiae.

The method developed here is applicable to measuring the inhibitory or stimulatory global effects of any treatment on rickettsial macromolecular synthesis. The specific activity approach eliminates a major source of artifacts that occur in working with obligate intracellular parasites, the inability to guarantee the reproducible recovery of whole parasites and parasitic macromolecules. However, the mechanism(s) by which IFN-γ inhibits rickettsial growth remains unknown. This study represents a beginning in the efforts to establish the nature of the biochemical lesions occurring within rickettsiae as their growth is inhibited in IFN-γ-treated cells.

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