Intramacrophage Passage of *Mycobacterium tuberculosis* and *M. avium* Complex Alters the Drug Susceptibilities of the Organisms as Determined by Intracellular Susceptibility Testing Using Macrophages and Type II Alveolar Epithelial Cells

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*Mycobacterium tuberculosis* and *M. avium* complex strains given intramacrophage passage (I-type) were compared with those cultured in a liquid medium (E-type) for their drug susceptibilities when they were replicating in Mono-Mac-6 macrophages or A-549 cells. Their intracellular susceptibilities to rifalazil, clarithromycin, and levofloxacin were decreased more in I-type organisms than in E-type organisms, except that their rifalazil susceptibility inside A-549 cells was markedly increased in I-type organisms.

*Mycobacterium avium* complex (MAC) adapted to intracellular milieu by intramacrophage passage (I-type) is known to display increased efficiency in invading other macrophages compared to extracellularly grown MAC (E-type) (2). It is also reported that *Mycobacterium tuberculosis* obtained by intramacrophage passage (I-type) more efficiently invades type II pneumocytes, one of the major portals of mycobacterial entry to the lungs (1, 7, 9, 10), and exerts greater cytotoxicity than extracellularly passed *M. tuberculosis* (E-type) (6). It thus appears that the mode of interaction of I-type mycobacteria with macrophages and type II pneumocytes is different from that of E-type organisms. Therefore, we examined the intracellular susceptibilities of I-type and E-type mycobacteria to some antimicrobials, including rifalazil (RLZ) (3, 11), clarithromycin (CLR), and levofloxacin (LVX), when these organisms were residing within macrophages or type II pneumocytes.

*M. tuberculosis* Kurono and MAC N-444 (*M. avium*, serovar 8) strains, grown in 7H9 broth or passed through Mono-Mac-6 (MM6) human monocytic cells (12) for 5 days, were used as E-type or I-type organisms, respectively. The intracellular susceptibilities to the test drugs of these organisms inside MM6 macrophages or A-549 human type II alveolar cells were measured as follows (10). First, MM6 macrophages cultured in RPMI 1640 medium (RPMI) containing 5% fetal bovine serum (FBS) were infected with *M. tuberculosis* (multiplicity of infection [MOI] = 3) or MAC (MOI = 10) at 37°C in a CO2 incubator for 4 h. After being washed with 2% FBS–Hanks’ balanced salt solution by centrifugation (150 × g, 5 min), infected cells (4 × 10^5) were cultivated in 1% FBS–RPMI (0.2 ml) in the presence or absence of test drugs at the maximum concentration of drug in serum (C_max) (Fig. 1). At intervals, the cells were lysed with 0.07% sodium dodecyl sulfate, subsequently neutralized with 6% bovine serum albumin, and released organisms were collected and washed with distilled water by centrifugation (2,000 × g, 30 min). The CFU of recovered organisms were counted on 7H11 agar plates. Second, A-549 cells (4 × 10^6) cultivated in 5% FBS–Ham’s F-12K medium were infected with *M. tuberculosis* (MOI = 3) or MAC (MOI = 10) at 37°C for 3 h. After being washed with 2% FBS–HBSS, infected cells were cultured in 0.2 ml of 1% FBS–F-12K medium in the presence or absence of test drugs at C_max. At intervals, a CFU counting assay was done as described above.

I/E[inv], the ratio of invasiveness of I-type organisms to that of E-type organisms, was calculated as (intracellular CFU of I-type organisms after infection)/(intracellular CFU of E-type organisms after infection). I/E[growth], the ratio of intracellular growth of I-type organisms to that of E-type organisms, was calculated as (∆CFU of I-type organisms during a 5-day cultivation)/(∆CFU of E-type organisms during a 5-day cultivation). I/E[Drug-S], the ratio of the decrease in residual CFU of I-type organisms due to the activity of test antimicrobial agents to that of E-type organisms, was calculated as [CFU_I-type (−drug)/CFU_I-type (+drug)]/[CFU_E-type (−drug)/CFU_E-type (+drug)].

Figure 1 shows the antimicrobial effects of test drugs against E-type and I-type mycobacteria residing within MM6 macrophages. First, I/E[inv] values of *M. tuberculosis* and MAC were 0.79 and 4.17, respectively. Thus, invasiveness of *M. tuberculosis* into MM6 macrophages was decreased by intramacrophage passage, whereas the opposite result was obtained for MAC. I/E[growth] values of *M. tuberculosis* and MAC were 2.81 and 2.29, respectively, indicating that their ability to replicate within MM6 macrophages was increased by intramacrophage passage. Second, RLZ and LVX exhibited CFU-reducing activity, and CLR caused growth inhibition of both types of *M. tuberculosis*. Notably, the intracellular susceptibility of I-type *M. tuberculosis* to these drugs was somewhat lower than that of E-type *M. tuberculosis*: the mean values of I/E[Drug-S] on days 5 and 7 were 0.85, 0.48, and 0.57 for RLZ, CLR, and LVX, respectively. Third, RLZ reduced bacterial CFU of both types of MAC. CLR also reduced bacterial CFU of E-type MAC, but it caused only growth inhibition of I-type MAC. LVX showed...
weak antimicrobial action against them. In most cases, the
intramacrophage drug susceptibility of I-type MAC was some-
what lower than that of the E-type MAC: I/E[Drug-S] values
were 0.95, 0.71, and 0.71 for RLZ, CLR, and LVX, respectively.
Figure 2 shows the results of the same experiment with
A-549 cells. First, I-type organisms were more ef
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cient than
E-type organisms in invading A-549 cells: I/E[inv] values of
M. tuberculosis
and MAC were 2.51 and 4.47, respectively. The
ability of M. tuberculosis
but not MAC to replicate inside A-549
cells was increased by intramacrophage passage: I/E[growth]
values of M. tuberculosis
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both types of M. tuberculosis. LVX exerted CFU-reducing ac-
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inhibition of both types of M. tuberculosis. Third, CLR exhib-
ted CFU-reducing activity against both types of MAC, while
RLZ caused growth inhibition of them. LVX showed weak
inhibitory action against both types of MAC. Fourth, intracel-
lar RLZ susceptibilities of I-type M. tuberculosis and MAC inside A-549 cells were markedly increased compared to those
of E-type organisms. In contrast, the susceptibilities of I-type
M. tuberculosis and MAC to CLR and LVX were lower than
those of E-type organisms: I/E[Drug-S] values of M. tuberculosis
were 7.15, 0.77, and 0.31 for RLZ, CLR, and LVX, respec-
tively, and those of MAC were 8.70, 0.61, and 0.90 for
these drugs, respectively.
Concerning the present findings, the following discussion
can be made. First, this study indicated that the intracellular drug susceptibility of I-type MAC was somewhat lower than that of the E-type MAC: I/E[Drug-S] values
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Phagocyte cell membrane via different types of receptors (complement receptors, mannose receptor, vitronectin receptor, β1-integrins, etc.) (4). Intracellular signalling pathways initiated by the ligation of such receptors may differentially influence the maturation of phagosomes and phagolysosomes containing mycobacteria and may regulate the mode of fusion of phagosomal vesicles with the early or late endosomes and lysosomes. Therefore, the difference in the types of macrophage receptors, which are used for mycobacterial entry into macrophages, between I-type and E-type *M. tuberculosis* or MAC may result in differential efficiencies in the delivery of extracellular antimicrobial drugs into the phagosomes containing these mycobacteria. This hypothesis is being verified by electron microscopy studies of the intracellular delivery of drugs in MM6 macrophages and A-549 cells engulfing the I-type and E-type mycobacteria.

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REFERENCES


