INFECTION AND IMMUNITY, May 1999, p. 2117–2124 Vol. 67, No. 5
0019-9567/99/$04.00+0

Legionella pneumophila Utilizes the Same Genes To Multiply within *Acanthamoeba castellanii* and Human Macrophages

GIL SEGAL AND HOWARD A. SHUMAN*

Department of Microbiology, College of Physicians & Surgeons, Columbia University, New York, New York 10032

Received 11 November 1998/Returned for modification 15 January 1999/Accepted 28 January 1999

In previous reports we described a 22-kb *Legionella pneumophila* chromosomal locus containing 18 genes. Thirteen of these genes (*icmT*, *-R*, *-Q*, *-P*, *-O*, *-M*, *-L*, *-K*, *-E*, *-C*, *-D*, *-J*, and *-B*) were found to be completely required for intracellular growth and killing of human macrophages. Three genes (*icmS*, *-G*, and *-F*) were found to be partially required, and two genes (*lphA* and *tphA*) were found to be dispensable for intracellular growth and killing of human macrophages. Here, we analyzed the requirement of these genes for intracellular growth in the protozoan host *Acanthamoeba castellanii*, a well-established important environmental host of *L. pneumophila*. We found that all the genes that are completely required for intracellular growth in human macrophages are also completely required for intracellular growth in *A. castellanii*. However, the genes that are partially required for intracellular growth in human macrophages are completely required for intracellular growth in *A. castellanii*. In addition, the *lphA* gene, which was shown to be dispensable for intracellular growth in human macrophages, is partially required for intracellular growth in *A. castellanii*. Our results indicate that *L. pneumophila* utilizes the same genes to grow intracellularly in both human macrophages and amoebae.

Legionella pneumophila, the causative agent of Legionnaires' disease, is a broad-host-range facultative intracellular pathogen. The bacteria are able to infect, multiply within, and kill human macrophages, as well as free-living amoebae (29, 41). *L. pneumophila* infection can be divided into several steps that occur in similar ways in both hosts. The bacteria are taken up by regular phagocytosis or by a special mechanism termed "coiling" phagocytosis (12, 27); the bacteria are then found within a specialized phagosome that does not fuse with lysosomes (12, 26). The specialized phagosome undergoes several recruitment events that include association with smooth vesicles, mitochondria, and rough endoplasmic reticulum (1, 25, 49). The bacteria multiply within the specialized phagosome until the cell eventually lyses, releasing bacteria that can start new rounds of infection (29, 41).

Two regions of genes required for human macrophage killing and intracellular multiplication have been discovered in *L. pneumophila* (reviewed in reference 45). Region I contains 7 genes (*icmV*, *-W*, and *-X* and *dotA*, *-B*, *-C*, and *-D*) (10, 13, 32, 50), and region II contains 16 genes (*icmT*, *-S*, *-R*, *-Q*, *-P*, *-O*, *-M*, *-L*, *-K*, *-E*, *-G*, *-C*, *-D*, *-J*, *-B*, and *-F*) (3, 40, 43, 44, 50). The role of these genes in *L. pneumophila*'s ability to grow intracellularly in amoebae had not previously been determined. In other studies, transposon mutagenesis of the *L. pneumophila* genome identified mutants defective for intracellular growth and killing of both human macrophages and protozoa (23), as well as other mutants found to be defective for intracellular growth only in human macrophages (24) or only in protozoa (15). However, the genes disrupted in these mutants were not described.

The aim of this study was to determine if the *icm* genes listed above are also required for intracellular growth in protozoa. One hypothesis is that *icm* genes are specifically required for intracellular growth in human macrophages. An alternative hypothesis is that *icm* genes are required for intracellular growth in both human macrophages and protozoan hosts. The results presented here clearly show that *icm* genes are required for intracellular growth in both hosts, thus supporting the second hypothesis.

* Corresponding author. Mailing address: Department of Microbiology, College of Physicians & Surgeons, Columbia University, 701 West 168th St., New York, NY 10032. Phone: (212) 305-6913. Fax: (212) 305-7323. E-mail: shuman@cuccfa.ccc.columbia.edu.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Bacterial strains and plasmids used in this work are described in Table 1 and Table 2, respectively. Bacterial media, plates, and antibiotic concentrations were used as described before (44).

Plasmid construction. The cloning vectors pMMB207 (35) and pMMB207αb-Kn-14 (46) were used to construct a new *L. pneumophila* cloning vector, pMMB207-Kn-14. Both pMMB207 and pMMB207αb-Kn-14 were digested with *Bsp*EI and *Mlu*I. The *Bsp*EI-*Mlu*I fragment of pMMB207αb-Kn-14 containing the Kn insertion in *mobA* was cloned into pMMB207 to generate pMMB207-

TABLE 1. *L. pneumophila* strains

Strain	Genotype and features	Reference
25D	Icm$^-$ avirulent mutant	28
GS3001	JR32 *icmS3001*::Kan	44
GS3002	JR32 *icmP3002*::Kan	44
GS3003	JR32 *icmO3003*::Kan	44
GS3005	JR32 *icmO-icmN3005*::Kan	43
GS3006	JR32 *icmN3006*::Kan	43
GS3007	JR32 *icmN3007*::Kan	43
GS3008	JR32 *icmM3008*::Kan	43
GS3009	JR32 *icmL3009*::Kan	43
GS3010	JR32 *icmK3010*::Kan	43
GS3011	JR32 *icmT3011*::Kan	46
JR32	Salt-sensitive isolate of AM511	42
LELA1275	JR32 *icmF1275*::Tn*903*dII*lacZ*	42
LELA3118	JR32 *dotA3118*::Tn*903*dII*lacZ*	42
LELA3244	JR32 *icmD3244*::Tn*903*dII*lacZ*	42
LELA3393	JR32 *icmB3393*::Tn*903*dII*lacZ*	42
LELA3463	JR32 *icmQ3463*::Tn*903*dII*lacZ*	42
LELA3473	JR32 *icmR3473*::Tn*903*dII*lacZ*	42
LELA4004	JR32 *icmX4004*::Tn*903*dII*lacZ*	42
LELA4432	JR32 *icmE4432*::Tn*903*dII*lacZ*	42
MW627	JR32 *tphA627*::Kan	40
MW635	JR32 *icmG635*::Kan	40
MW645	JR32 *icmC645*::Kan	40
MW656	JR32 *icmJ656*::Kan	40

TABLE 2. Plasmids used in this study

Plasmid	Features	Reference or source
pGS-Lc-34-14	*icmPO* in pMMB207αb-Km-14	46
pGS-Lc-34-D1	*icmO* in pMMB207αb	44
pGS-Lc-37-14	*icmTS* in pMMB207αb-Km-14	46
pGS-Lc-37-D1	*icmS* in pMMB207αb	44
pGS-Lc-47	*icmNMLKEG* in pMMB207αb	43
pGS-Lc-55-14	*icmF* and *tphA* in pMMB207αb-Km-14	This study
pGS-Lc-63-14	*icmGCD* in pMMB207-Km-14	This study
pMMB207	RSF1010 derivative, *IncQ lacI*^{q} Cm^r P*tac oriT*	35
pMMB207-Km-14	pMMB207 with *mobA*::Km	This study
pMMB207αb-Km-14	pMMB207αb with *mobA*::Km	46
pMW-100	*icmGCDJB*, *tphA*, and *icmF* in pMMB207	40
pMW-275	*icmEGCDJB*, *tphA*, and *icmF* in pLAFR1	43
pMW-560	*icmB* in pBC-SK+	40
pMW-604	*icmGCD* in pMMB207	40

Kn-14. Both the pMMB207-Kn-14 and pMMB207αb-Kn-14 vectors contain a Kn insertion in *mobA*.

To construct a *mobA*-less complementing plasmid for the *icmGCD* region, a *Bam*HI-*Eco*RI fragment containing these genes was cloned from pMW604 (40) into the same sites in pMMB207-Kn-14 to generate pGS-Lc-63-14.

The cosmid pMW-275 (43) was used to construct a complementing plasmid for the *icmF-tphA* region. A *Xho*I 9-kb fragment was filled in and subcloned into the *Sma*I site of pMMB207αb-Kn-14 to generate pGS-Lc-55-14; this plasmid contains the *icmF* and *tphA* genes and about 4 kb of DNA upstream of *icmF*.

Intracellular growth in HL-60-derived macrophages. Intracellular growth assays were performed as previously described (46), with the following modifications. Wells of a 24-well microtiter dish containing 3×10^6 differentiated HL-60-derived macrophages were used for infection. *L. pneumophila* was added to the wells at a multiplicity of infection of approximately 0.1, and the infected HL-60-derived macrophages were incubated for 1 h at 37°C under CO_2 (5%). Then the wells were washed three times with RPMI containing glutamine, and 0.6 ml of RPMI medium, containing 2 mM Gln and 10% normal human serum, was added to the wells. The supernatant of each well was sampled at intervals of about 12 h, and numbers of CFU were determined by plating samples on ACES [*N*-(2-acetamido)-2-aminoethanesulfonic acid]-buffered charcoal-yeast extract (ABCYE) plates.

Intracellular growth in *Acanthamoeba castellanii*. *A. castellanii* (ATCC 30234) was grown in 30 ml of proteose peptone-yeast extract-glucose medium (PYG) media (34) in a 75-cm^2 tissue culture flask at 28°C, as adherent cells, until confluence was reached. Before starting an experiment, the flask was gently shaken and the PYG containing nonadherent amoebae was removed. New PYG was added to the flask and the amoebae were taken off by tapping the flask sharply. The resulting suspension was centrifuged for 10 min at $220 \times g$, the amoebae were resuspended in PYG at a concentration of 3×10^5 amoebae/ml, and 0.5 ml of the suspension was added to each well of a 48-well plate (1.5×10^5 amoebae/well). The amoebae were incubated for 1 h at 37°C to let the amoebae adhere. Then the PYG was aspirated, the wells were washed once with 0.5 ml of warm (37°C) Ac buffer (34), and 0.5 ml of warm Ac buffer was added to each well. *L. pneumophila*, in Ac buffer, was added to the wells at a multiplicity of infection of approximately 1. The plate was incubated for 30 min at 37°C, then the Ac buffer was aspirated, the wells were washed three times with 0.5 ml of warm Ac buffer, and 0.6 ml of warm Ac buffer was added to each well. Fifty-microliter samples were taken out at intervals of about 12 h, and numbers of CFU were determined by plating samples on ABCYE plates.

RESULTS

All *icm* genes completely required for intracellular growth in HL-60-derived macrophages are completely required for intracellular growth in *A. castellanii*. Mutants containing insertions in 13 *icm* genes (*icmT*, *-R*, *-Q*, *-P*, *-O*, *-M*, *-L*, *-K*, *-E*, *-C*,

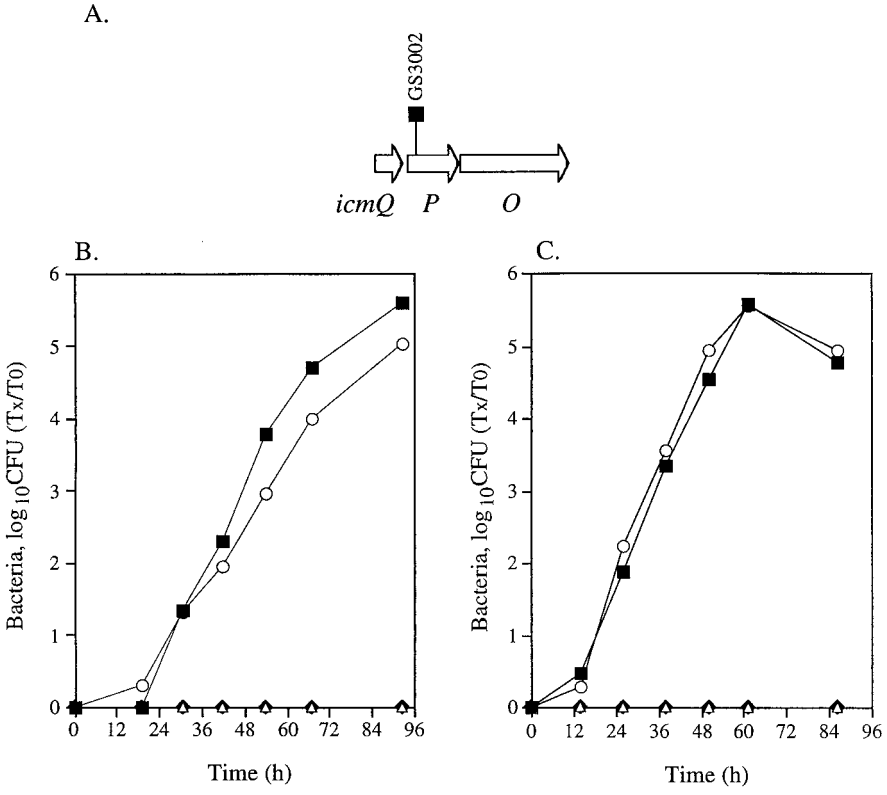

FIG. 1. Intracellular growth of an *icmP* insertion mutant in HL-60-derived macrophages and *A. castellanii*. (A) Chromosomal arrangement of the region surrounding *icmP*. The location of the insertion in *icmP* (GS3002) is shown. Intracellular growth in HL-60-derived macrophages (B) and in *A. castellanii* (C) was tested as described in Materials and Methods; the experiments were done at least three times, and results similar to those shown were obtained. ■, JR32; ◆, 25D; △, GS3002 containing pMMB207αb-Km-14; ○, GS3002 containing pGS-Lc-34-14.

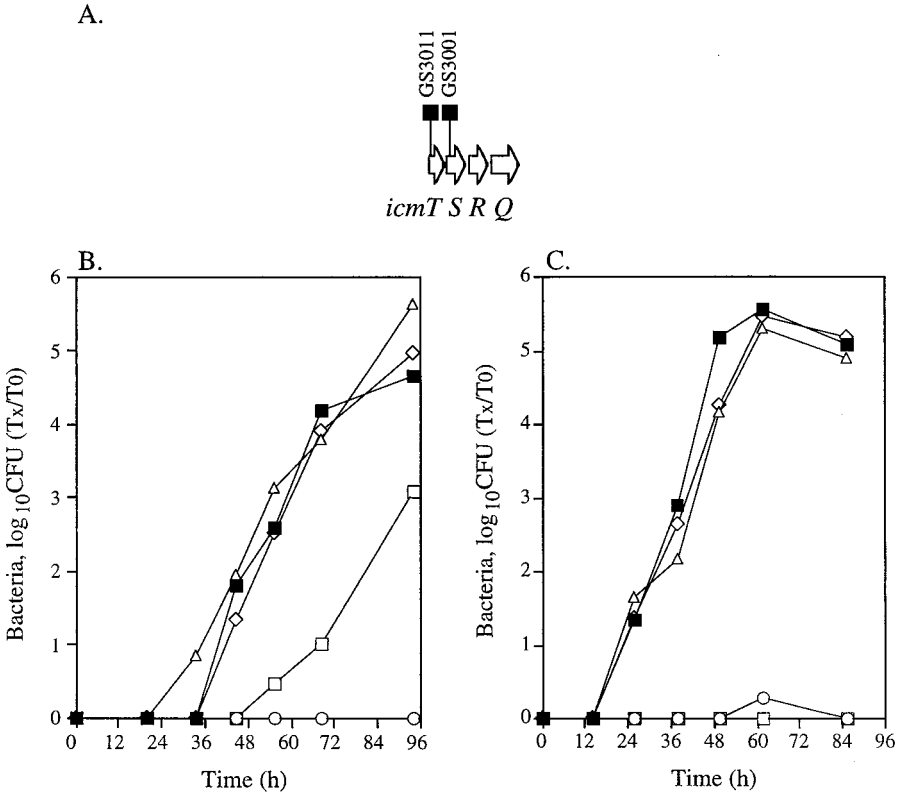

FIG. 2. Intracellular growth of *icmT* and *icmS* insertion mutants in HL-60-derived macrophages and *A. castellanii*. (A) Chromosomal arrangement of the region surrounding *icmS*. The locations of the deletion substitutions (GS3001 and GS3011) are shown. Intracellular growth in HL-60-derived macrophages (B) and in *A. castellanii* (C) was tested as described in Materials and Methods; the experiments were done at least three times, and results similar to those shown were obtained. ■, JR32; ○, GS3011 containing pMMB207αb-Km-14; △, GS3011 containing pGS-Lc-37-14; □, GS3001 containing pMMB207αb-Km-14; ◇, GS3001 containing pGS-Lc-37-14.

-D, *-J*, and *-B*) located in region II that were found to be completely required for killing of HL-60-derived macrophages (40, 43, 44) were analyzed for their ability to grow inside HL-60-derived macrophages and *A. castellanii*. An example of such a mutant that contains an insertion in *icmP* (GS3002) is presented in Fig. 1. As can be seen in Fig. 1B and C, a mutant containing an insertion in *icmP* was found to be completely defective for intracellular growth in both hosts, and its growth reached wild-type levels when a plasmid containing the *icmP* and *icmO* genes (pGS-Lc-34-14) was introduced into it. A similar analysis was done with mutants with insertions in all the *icm* genes mentioned (*icmT*, *-R*, *-Q*, *-P*, *-O*, *-M*, *-L*, *-K*, *-E*, *-C*, *-D*, *-J*, and *-B*), and a result similar to the one presented for the *icmP* insertion mutant was obtained (the mutants tested are listed in Table 1). All the genes that were found to be completely required for killing of HL-60-derived macrophages were found to be also completely required for intracellular growth in these cells, as well as for intracellular growth in *A. castellanii*. The mutants with insertions in *icmT*, *icmP*, and *icmJ* (GS3011, GS3002, and MW656, respectively) were also tested for intracellular growth when the downstream genes (*icmS*, *icmO*, and *icmB*, respectively) that probably form one transcriptional unit with them were expressed from a plasmid (pGS-Lc-37-D1, pGS-Lc-34-D1, and pMW-560, respectively). No intracellular growth was observed with these mutants when the downstream gene was supplied, indicating that these genes by themselves are required for intracellular growth. The mutant containing an insertion in *icmC* (MW645) was only partially complemented when the plasmid pGS-Lc-63-14 was introduced into it (see Fig. 4). Similar results were obtained when mutants containing insertions in *icmM*, *-L*, *-K*, and *-E* were complemented with the plasmid pGS-Lc-47. The reason for the partial complementation is not known. Two additional genes (*icmX* and *dotA*) located in region I (45) that were shown to be required for human macrophage killing (10, 13) were also found to be required for intracellular growth in *A. castellanii*.

The *icmS* gene. In a previous report (44), we showed that the *icmT* and *icmS* genes probably form one transcriptional unit and that the downstream *icmR* gene (Fig. 2A) is probably transcribed individually. In addition, the mutant containing an insertion in *icmS* (GS3001) was shown to retain some ability to kill HL-60-derived macrophages. Here, we compared the intracellular growth of the *icmS* insertion mutant in both HL-60-derived macrophages and *A. castellanii*, and the results are presented in Fig. 2B and C, respectively. The *icmS* insertion mutant was found to be only partially defective for intracellular growth in HL-60-derived macrophages (Fig. 2B) but completely defective for intracellular growth in *A. castellanii*. A mutant containing an insertion in *icmT* (GS3011) was found to be completely defective for growth in both hosts (Fig. 2B and C). Both insertion mutants (GS3001 and GS3011) attained wild-type levels when supplemented with a plasmid containing the *icmT* and *icmS* genes (pGS-Lc-37-14).

The *lphA* (*icmN*) gene. The *lphA* gene (*lphA* stands for lipoprotein homolog) was found to be dispensable for killing of HL-60-derived macrophages (43). Because this gene is located in the middle of a region containing genes required for intracellular growth (Fig. 3A), this result was surprising. Therefore, we used three different insertion mutants (GS3005, GS3006, and GS3007) to analyze this gene (Fig. 3A). Mutants contain-

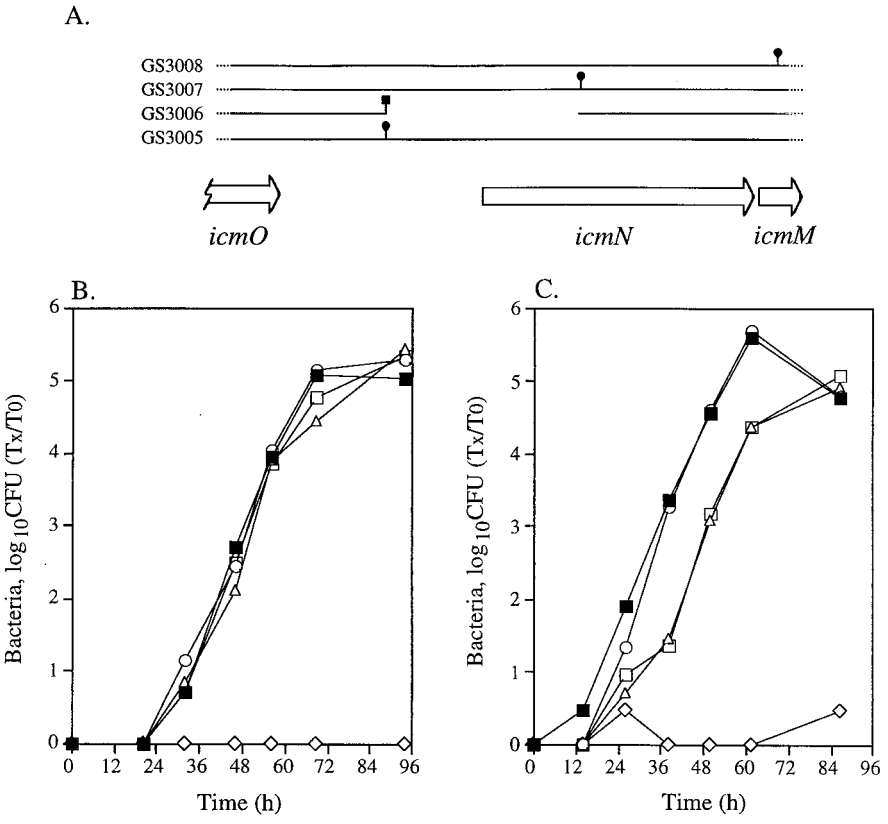

FIG. 3. Intracellular growth of *icmN* and *icmM* insertion mutants in HL-60-derived macrophages and *A. castellanii*. (A) Chromosomal arrangement of the region surrounding *icmN*. The chromosomal arrangements of the mutants tested are shown above the genes. Intracellular growth in HL-60-derived macrophages (B) and in *A. castellanii* (C) was tested as described in Materials and Methods; the experiments were done at least three times, and results similar to those shown were obtained. ■, JR32; ○, GS3005, □, GS3006, △, GS3007; ◇, GS3008.

ing an insertion (GS3007) or a deletion substitution (GS3006) in *lphA* (Fig. 3A) were found to have no defect in intracellular growth in HL-60-derived macrophages (Fig. 3B); this result agreed with the levels of cytotoxicity obtained with these mutants (43). When these mutants were analyzed for their intracellular growth in *A. castellanii* (Fig. 3C), a moderate defect in intracellular growth was observed. Growth was reduced by a factor of up to 100 at 40 to 50 h postinfection, in comparison to the wild-type strain (JR32). Due to the weak phenotype, we tested an additional mutant containing an insertion in the region between *icmO* and *lphA* (GS3005) (Fig. 3A); this mutant was found to be identical to the wild-type strain in its ability to grow intracellularly. Because *lphA* insertion mutants are defective for intracellular growth in *A. castellanii*, we renamed this gene *icmN*.

We tried to complement the *icmN* mutants with plasmids containing *icmN* or *icmN*, *-M*, *-L*, *-K*, and *-E*, but we were unable to observe complementation. It is very unlikely that the phenotype of the *icmN* insertion mutants is due to polarity on the downstream gene *icmM* (Fig. 3A), because a mutant containing an insertion in *icmM* (GS3008) was found to be completely defective for intracellular growth in both hosts (Fig. 3B and C). We assume that if the phenotype of the *icmN* mutants was due to polarity on *icmM*, we would have observed a reduction in the intracellular growth of the *icmN* mutants in both hosts and not only in *A. castellanii*.

The *icmG* gene. An *icmG* insertion mutant (MW635) was shown to be moderately defective in killing HL-60-derived macrophages, and the two genes located downstream of *icmG* (*icmC* and *icmD*) (Fig. 4A) were shown to be completely required for killing of HL-60-derived macrophages (40). When the *icmG* insertion mutant was tested for intracellular growth in HL-60-derived macrophages (Fig. 4B), it was found to have a weak reduction in comparison to the wild-type strain (JR32). When this mutant was tested for its ability to grow inside *A. castellanii*, no growth was observed (Fig. 4C). When a plasmid containing *icmG*, *-C*, and *-D* (pGS-Lc-63-14) was introduced into this mutant, only partial complementation was observed (Fig. 4B and C). A mutant containing an insertion in *icmC* (MW645), located downstream from *icmG*, was found to be completely defective for intracellular growth in both hosts (Fig. 4B and C). As was described for the mutant with the insertion in *icmG*, the *icmC* insertion mutant was only partially complemented with pGS-Lc-63-14. No increase in complementation efficiency of either mutant was observed when a plasmid (pMW-100) containing additional downstream genes was used for complementation. The reason for the partial complementation is not known.

The *icmF* and *tphA* genes. The *icmF* and *tphA* genes are located on the opposite strand in relation to the other genes found in region II (Fig. 5A). When mutants containing insertions in *icmF* and *tphA* (*tphA* stands for transport protein homolog) were tested for their ability to kill HL-60-derived macrophages, it was found that a mutant containing an insertion in *icmF* was partially defective for killing HL-60-derived macrophages, and a mutant containing an insertion in *tphA* (MW627) was not defective in killing HL-60-derived macrophages (40). Here, we tested mutants with insertions in these

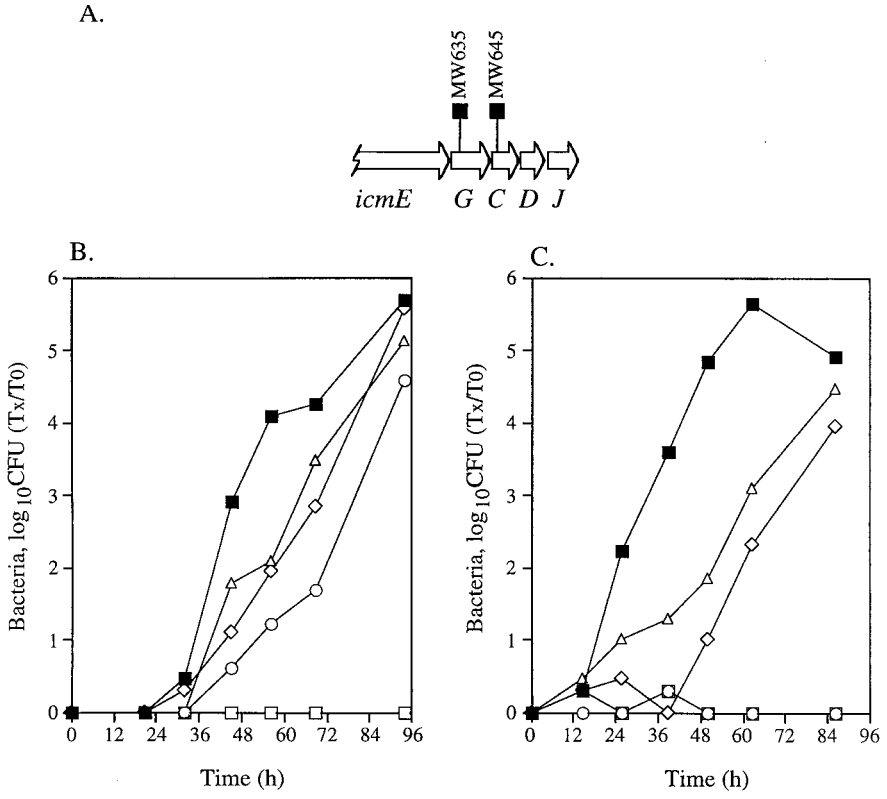

FIG. 4. Intracellular growth of *icmG* and *icmC* insertion mutants in HL-60-derived macrophages and *A. castellanii*. (A) Chromosomal arrangement of the region surrounding *icmG*. The locations of the deletion substitutions (MW635 and MW645) are shown. Intracellular growth in HL-60-derived macrophages (B) and in *A. castellanii* (C) was tested as described in Materials and Methods; the experiments were done at least three times, and results similar to those shown were obtained. ■, JR32; ○, MW635 containing pMMB207-Km-14; △, MW635 containing pGS-Lc-63-14; □, MW645 containing pMMB207-Km-14; ◇, MW645 containing pGS-Lc-63-14.

genes (LELA1275 for *icmF* and MW627 for *tphA*) for intracellular growth in HL-60-derived macrophages (Fig. 5B) and *A. castellanii* (Fig. 5C). The results obtained with HL-60-derived macrophages are consistent with the observation made with the cytotoxicity assays (40); the *icmF* insertion mutant was found to be weakly defective for growth inside HL-60-derived macrophages, and the *tphA* insertion mutant was found to be identical to the wild-type strain. In contrast, the mutant with the insertion in *icmF* was completely defective for intracellular growth in *A. castellanii*, and the mutant with the insertion in *tphA* was indistinguishable from the wild-type strain (Fig. 5C). The mutant with the insertion in *icmF* (LELA1275) achieved a wild-type level of growth when a plasmid containing *icmF* and *tphA* (pGS-Lc-55-14) was introduced into the mutant (Fig. 5B and C).

DISCUSSION

L. pneumophila is a broad-host-range facultative intracellular pathogen that overcomes many natural host defense mechanisms, enabling it to cause disease in humans. Like *Mycobacterium tuberculosis* (4), *Chlamydia psittaci* (22), and *Toxoplasma gondii* (30), *L. pneumophila* multiplies within human cells inside a specialized vacuole that does not fuse with secondary lysosomes (26). In nature, *L. pneumophila* uses a similar mechanism to infect and multiply within free-living amoebae (1, 12, 19).

Besides *Legionella*, several other bacterial species, such as *Mycobacterium avium*, *Chlamydia pneumoniae*, and *Listeria monocytogenes*, were shown to survive and multiply in amoebae (17, 18, 31). For *Legionella*, growth within amoebae and ciliated protozoa is probably the main means of survival and multiplication in the environment (5, 6, 19). *Legionella* has been shown to multiply intracellularly in several species of protozoa, such as *Hartmannella*, *Acanthamoeba*, *Naegleria*, and *Tetrahymena* (20, 21, 36, 41). During outbreaks of Legionnaires' disease, the water sources for *L. pneumophila* were usually found to contain amoebae and/or protozoa (5, 19). Intracellular growth in amoebae, in comparison to growth on artificial media, was shown to affect *L. pneumophila* in several ways. It was shown to enhance invasion into monocytic cells (34), cause changes in bacterial cell surface properties (8), and increase bacterial resistance to antibiotics (9) and bacterial susceptibility to chemicals (7). In addition, viable but nonculturable *L. pneumophila* can be resuscitated by coculture with protozoa (47). Moreover, coinfection of mice with *L. pneumophila* and *Hartmannella* was shown to cause a more severe respiratory disease than infection with either organism alone (14, 15). The finding that *A. castellanii* can form respirable vesicles in which *L. pneumophila* can survive (11) suggests that the vesicles might serve as one of the ways in which *L. pneumophila* can enter human lungs. These results indicate that the ability of *L. pneumophila* to multiply within protozoan hosts plays a critical role in its survival in the environment and its ability to cause disease in humans.

L. pneumophila infection of human monocytes and amoebae was studied intensively in the early 1980s by Horwitz and Silverstein (29), and Rowbotham (41). Further studies of *L. pneumophila* infection revealed that the process of infection occurs in very similar ways in both hosts (1, 12, 26, 27, 49). Several

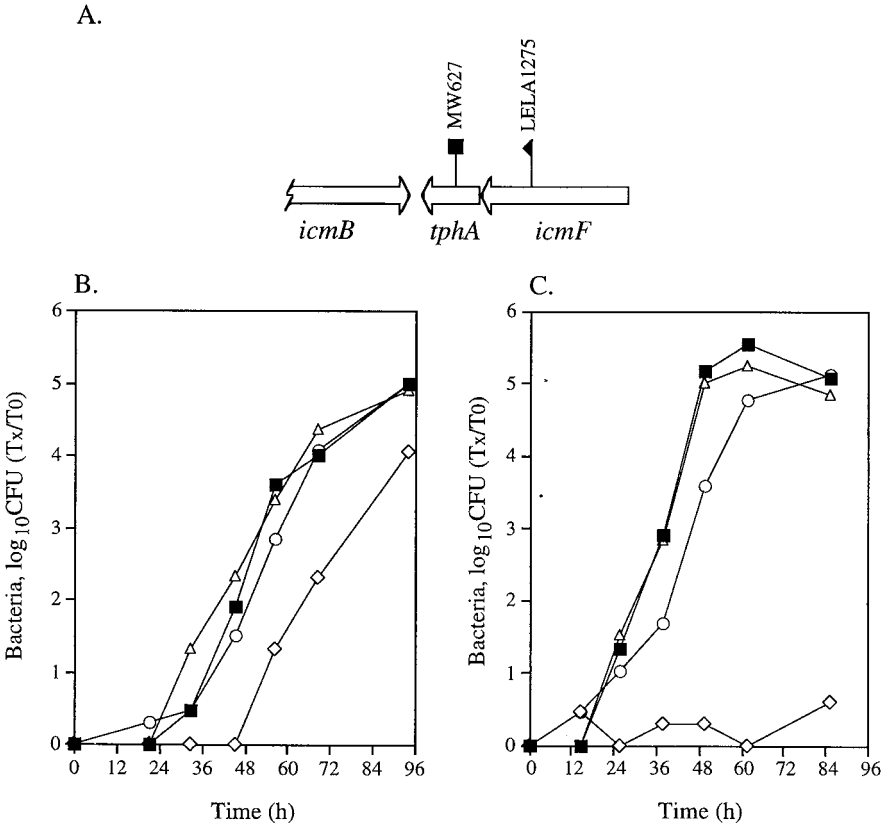

FIG. 5. Intracellular growth of *icmF* and *tphA* insertion mutants in HL-60-derived macrophages and *A. castellanii*. (A) Chromosomal arrangement of the region surrounding *icmF*. The locations of the insertions (LELA1275 and MW627) are shown. Intracellular growth in HL-60-derived macrophages (B) and in *A. castellanii* (C) was tested as described in Materials and Methods; the experiments were done at least three times, and results similar to those shown were obtained. ■, JR32; ◇, LELA1275 containing pMMB207αb-Km-14; ○, LELA1275 containing pGS-Lc-55-14; △, MW627.

groups compared the requirement of different genes for and the fate of different mutants in intracellular growth in human monocytes and amoebae. Most of the genes tested (*gspA*, *pilEL*, *hpb*, *lly*, and *msp*) were found to be dispensable for growth in both hosts (2, 33, 37, 48, 51); the *mip* gene was shown to be moderately attenuated for growth in both hosts (16). In contrast, mutants that were tested for growth in both hosts can be separated into three groups: A, mutants attenuated for growth in both hosts (23, 38, 39); B, mutants attenuated for growth only in human monocytes (24); and C, mutants attenuated for growth only in amoebae (15). However, the genes disrupted in the mutants from these three groups were not described.

Studies performed in our lab and in the Isberg lab yielded information about several *icm* and *dot* genes that were shown to be required for intracellular growth and killing of human macrophages (reviewed in reference 45). The role of these genes in the ability of *L. pneumophila* to multiply in amoebae has not previously been examined. Here, we present a detailed analysis of the requirement of 18 genes located in *icm* and *dot* region II for *L. pneumophila* intracellular growth in *A. castellanii*; the data are summarized in Fig. 6. All the genes that were shown to be completely required for intracellular growth in human macrophages were also found to be completely required for intracellular growth in *A. castellanii*. However, all the genes that were shown to be partially required for intracellular

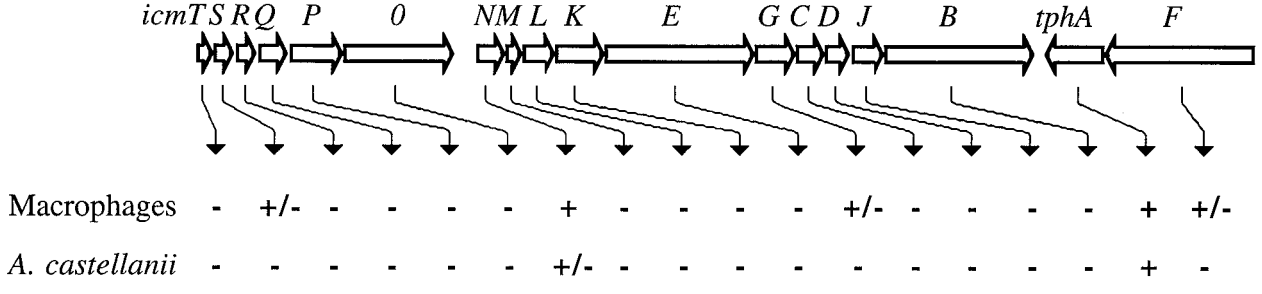

FIG. 6. Intracellular growth requirements for genes in region II. The first line under the genes indicates the growth phenotype in HL-60-derived macrophages, and the second line indicates the growth phenotype in *A. castellanii*. −, no intracellular growth was observed; +/−, partial intracellular growth was observed; +, intracellular growth was similar to that of the wild-type strain.

growth in human macrophages were found to be completely required for intracellular growth in *A. castellanii*. The *icmN* gene, which was shown to be dispensable for intracellular growth in human macrophages, is partially required for intracellular growth in *A. castellanii*. Our data indicate that *L. pneumophila* utilizes the same genes to grow intracellularly in both human macrophages and amoebae, two evolutionarily distinct hosts. The ability of *L. pneumophila* to infect and multiply inside human macrophages and amoebae in similar ways and by using the same genes indicates that amoebae and human macrophages have many similar properties that allow the bacteria to carry out their infection in both hosts.

ACKNOWLEDGMENTS

The work was supported by a grant from the NIH (AI23549). G. Segal was supported by a long-term fellowship from the EMBO and the Stephen A. Morse Fellowship from Departments of Microbiology and Medicine of Columbia University.

We are grateful to Carmen Rodriguez for excellent technical assistance during this work.

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Editor: P. J. Sansonetti