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# Hypoexpression of Major Histocompatibility Complex Molecules on *Legionella pneumophila* Phagosomes and Phagolysosomes

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***Legionella pneumophila* is a facultative intracellular pathogen that parasitizes host mononuclear phagocytes. Cell-mediated immunity is pivotal to host defense against *L. pneumophila*, and the infected host cell may play a central role in processing and presenting parasite antigens to lymphocytes mediating cell-mediated immune responses. However, in the case of *L. pneumophila* and intracellular parasites in general, little is known about the intracellular trafficking of parasite antigens, the influence of parasite infection on major histocompatibility complex (MHC) expression, or the relationship of MHC molecules to sites of parasite replication. To learn more about this, we have used flow cytometry to study the expression of HLA-DR by monocytes infected with *L. pneumophila* and cryosection immunogold electron microscopy to study the distribution of MHC class I and II molecules on *L. pneumophila* phagosomes. Flow cytometry analysis demonstrated that *L. pneumophila* infection has little effect on the overall expression of HLA-DR by monocytes. Cryosection immunogold studies revealed abundant staining for MHC class I and II molecules on the plasma membrane of infected monocytes but little or no staining on the membranes of mature *L. pneumophila* phagosomes. Cryosection immunogold studies of an avirulent mutant of *L. pneumophila* that, unlike the wild type, does not inhibit phagosome-lysosome fusion and subsequently survives but does not multiply in a phagolysosome yielded similar results. We have previously found that MHC class I and II molecules are excluded from nascent phagosomes during coiling and conventional phagocytosis. The present work demonstrates that MHC molecules do not accumulate appreciably in the *L. pneumophila* phagosome as it matures and at a point in the life cycle of the organism in which it is replicating and producing immunoprotective T-cell antigens. This suggests that *L. pneumophila* does not reside in a typical endosomal compartment in the host cell and that *L. pneumophila* antigens may encounter MHC molecules at extraphagosomal sites within the host cell.**

*Legionella pneumophila*, the etiologic agent of Legionnaires' disease, is a facultative intracellular pathogen that parasitizes host mononuclear phagocytes (23). Cell-mediated immunity plays a major role in the host defense against *L. pneumophila* (5–8, 17, 24, 29, 30), and humoral immunity plays only a minor role (25, 26). The host cell in *L. pneumophila* infection, the mononuclear phagocyte, is an antigen-presenting cell and thus may stimulate a cell-mediated immune response by presenting parasite antigens bound to major histocompatibility complex (MHC) molecules to immune T cells.

The pathway for processing and presentation of intracellular parasite antigens is not understood. Among other issues, the intracellular distribution of MHC molecules in host cells infected by intracellular parasites is not generally known. Antoine et al. (2) have studied the distribution of Ia (class II MHC) antigens in mouse macrophages infected with *Leishmania amazonensis*. They found that the percentage of *Leishmania* parasitophorous vacuoles staining positively for Ia by immunoperoxidase steadily increased from 34% at 2 h to 87% at 48 h after infection. Mature *Leishmania* parasitophorous vacuoles showed intense staining for class II MHC molecules. This study suggested that class II MHC molecules accumulating in the parasite vacuole may play a role in presentation of parasite antigens by the infected host cell.

Intracellular parasites are a phylogenetically diverse group, and they reside and multiply within different intracellular compartments. Thus, whereas *Leishmania* sp. multiplies within a highly acidified phagolysosome, *L. pneumophila* multiplies within a mildly acidified phagosome that does not fuse with lysosomes (1, 9, 19, 22). Whether physiological differences between parasites and their replicative vacuoles influence the distribution of MHC molecules on the vacuoles is not known.

The distribution of MHC molecules on mature *L. pneumophila* phagosomes has not been studied previously. However, in another study on the distribution of monocyte membrane molecules during phagocytosis, we observed that both MHC class I and class II molecules are excluded from nascent phagosomes during ingestion of *L. pneumophila*, which occurs by coiling phagocytosis (11, 20). Similarly, MHC class I and II molecules are excluded from nascent *Escherichia coli* phagosomes during conventional phagocytosis of this organism (11). Moreover, the concentration of MHC class I and II molecules on *L. pneumophila* phagosomes decreased further during the immediate postphagocytic period. This prompted us to investigate whether this situation persisted or whether MHC molecules accumulate on the phagosome as it matures, a process which involves the sequential interaction of the phagosome with host cell smooth vesicles, mitochondria, and ribosomes, until the ribosome-lined replicative vacuole is formed (18).

In this article, we have explored the distribution of MHC class I and class II molecules on mature *L. pneumophila* phagosomes. These phagosomes contain bacteria that are

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both replicating and releasing immunoprotective molecules (10). We have also examined the distribution of MHC molecules on phagolysosomes containing a mutant of *L. pneumophila* that fails to inhibit phagosome-lysosome fusion and cannot multiply in monocytes (21). We shall demonstrate that both MHC class I and class II molecules are sparse on *L. pneumophila* phagosomes and phagolysosomes. In contrast to the abundant staining for MHC molecules on the plasma membrane, there is a marked hypoexpression of both MHC class I and class II molecules on the phagosomal and phagolysosomal membranes of these monocytes.

## MATERIALS AND METHODS

**Reagents.** Mouse monoclonal antibodies were purchased from the following sources: anti-HLA-DR (immunoglobulin G2b [IgG2b], clone BL2) from AMAC (Westbrook, Maine), anti-MHC class II invariant chain (IgG1, clone LN2 [12, 35]) from ICN Immunobiologicals (Costa Mesa, Calif.), and anti-$\beta_2$-microglobulin (IgG1, clone BM-63) from Sigma Chemical Co. (St. Louis, Mo.). Isotypic mouse myeloma control proteins were obtained from Cappel Organon-Teknika (West Chester, Pa.). Goat anti-mouse IgG-gold conjugate (10 nm) and goat anti-mouse IgG-phycoerythrin conjugate were purchased from Sigma. Rabbit antibody to *L. pneumophila* (serogroup 1) fluorescein isothiocyanate (FITC) conjugate was purchased from SciMedix (Denville, N.J.). Ficoll-Hypaque was purchased from Pharmacia (Piscataway, N.J.); glutaraldehyde was from Polysciences (Warrington, Pa.); polyvinyl alcohol, polyvinylpyrrolidone, and paraformaldehyde were from Sigma. RPMI medium and Dulbecco's phosphate-buffered saline (PBS) were purchased from GIBCO Laboratories (Santa Clara, Calif.). Recombinant human gamma interferon (IFN-$\gamma$), containing $2.65 \times 10^4$ antiviral U/$\mu$g, was a gift from Genentech (San Francisco, Calif.).

**Bacteria.** *L. pneumophila*, Philadelphia 1 strain, was grown in embryonated hen eggs, harvested, tested for viability and contaminants, and stored at −70°C as described previously (23). The egg-yolk-grown *L. pneumophila* bacteria were cultured one time only on charcoal-yeast extract agar, harvested after 4 days of growth, and used immediately. *L. pneumophila* mutant 25D, a mutant of the Philadelphia 1 strain, was obtained and cloned as described previously (21) and stored at −70°C. The mutant bacteria were cultured on charcoal-yeast extract agar, harvested after 4 days of growth, and used immediately.

**Analysis of MHC expression by flow cytometry.** Human mononuclear cells were isolated from fresh heparinized blood by using Ficoll-Hypaque density gradients. Mononuclear cells ($3 \times 10^6$/ml) were plated in 75-cm$^2$ Falcon tissue culture flasks (12 ml per flask) in RPMI medium containing 10% autologous human serum. Monocytes were allowed to adhere to the plastic for 90 min at 37°C in 5% $CO_2$–95% air, washed three times with RPMI medium, and incubated with or without 50 U of IFN-$\gamma$ per ml in RPMI medium containing 10% autologous serum overnight. Monocyte monolayers were infected by incubating them with *L. pneumophila* ($2 \times 10^8$/ml) in RPMI medium containing 10% fresh autologous human serum for 90 min at 37°C on a rotating platform (100 rpm). The monocytes were washed three times with RPMI medium to remove nonadherent bacteria and incubated for an additional 22 h with or without 50 U of IFN-$\gamma$ per ml in RPMI medium containing 10% autologous serum. The infected monocytes were then fixed with 1% paraformaldehyde in Dulbecco's PBS without calcium or magnesium (3.0 mM KCl, 1.5 mM $KH_2PO_4$, 8.1 mM $Na_2HPO_4$, 0.137 M NaCl [pH 7.40]) containing 0.1% Tween 20 for 10 min at room temperature, washed twice with PBS, incubated with 10 mM glycine-HCl in PBS for 30 min to quench aldehyde groups, and scraped from the culture flasks with Costar cell scrapers into PBS containing 0.1% bovine serum albumin (BSA). The monocytes were pelleted by centrifugation at 1,000 rpm for 10 min and incubated in 50% normal goat serum in PBS for 30 min at 4°C to block nonspecific antibody binding in subsequent steps. The monocytes were then incubated with primary mouse monoclonal antibodies to HLA-DR (10 $\mu$g/ml) or with mouse myeloma isotypic control immunoglobulins (IgG2b) overnight at 4°C. The monocytes were washed three times in PBS, incubated in phycoerythrin-conjugated goat anti-mouse antibody (diluted 1:50 in PBS-BSA) for 90 min at room temperature, and washed three times. The monocytes were incubated with 10% normal rabbit serum in PBS to block nonspecific binding of rabbit antibody and then incubated with fluorescein-conjugated rabbit anti-*L. pneumophila* antibody (diluted 1:50) in PBS with 10% normal rabbit serum and 0.1% Tween 20 for 90 min at room temperature. The cells were washed three times, and dual-color fluorescence was analyzed by using a Becton Dickinson FACScan flow cytometer.

**Localization of MHC molecules by immunogold staining of cryosections.** The expression of monocyte MHC molecules was increased to facilitate study of their distribution by incubating them with IFN-$\gamma$. Human mononuclear cells were isolated as described above and plated in 3.5-cm-diameter polystyrene tissue culture wells ($3 \times 10^6$ cells per ml, 2.5 ml per well) in RPMI medium containing 10% autologous serum. Monocytes were allowed to adhere to the plastic for 90 min at 37°C in 5% $CO_2$–95% air, washed three times with RPMI medium, and incubated overnight with 50 U of IFN-$\gamma$ per ml in RPMI medium containing 10% autologous serum. Monocyte monolayers were infected by incubating them with *L. pneumophila* as described above. After 22 h, a time period sufficient for *L. pneumophila* to multiply extensively in monocytes but not to lyse them, the monocytes were fixed in 4% paraformaldehyde–0.01% glutaraldehyde in 0.15 M cacodylate buffer (pH 7.30) for 2 h at 4°C, washed with cacodylate buffer, incubated with 10 mM glycine-HCl in cacodylate buffer for 30 min to quench aldehyde groups, and scraped from the tissue culture wells with a rubber policeman into cacodylate buffer containing 0.1% BSA. The scraped cells were pelleted by centrifugation at 1,000 rpm for 10 min, resuspended in 0.15 M sodium cacodylate (pH 7.30) containing 6.25% acrylamide (prepared from 37.5:1 acrylamide-bisacrylamide stock) for 1 h at 4°C, pelleted by centrifugation at 1,000 rpm for 10 min, and resuspended in freshly prepared 6.25% acrylamide containing 0.13% (vol/vol) TEMED (*N,N,N',N'*-tetramethylene diamine) and 0.83 mg of ammonium persulfate per ml. The monocytes were pelleted again at 800 × *g* for 10 min at 4°C, and the acrylamide was allowed to polymerize for 2 h at 4°C. The cell pellets were trimmed with a razor blade, infiltrated with 20% polyvinylpyrrolidone in 2.3 M sucrose overnight at 4°C, and cryosectioned at −90°C. Sections were transferred to nickel grids and incubated with 50% normal goat serum and 0.1% fish skin gelatin in 0.9% NaCl in 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.40) for 1 h at 4°C to block nonspecific antibody binding. Sections were incubated with a 10-$\mu$g/ml concentration of mouse monoclonal primary immunoglobulin or control isotypic myeloma immunoglobulin diluted in HEPES buffer contain-

TABLE 1. Immunofluorescence flow cytometry analysis of HLA-DR expression by uninfected monocytes and monocytes infected with *L. pneumophila*

| Expt | Status of monocytes | IFN-γ | % Monocytes infected | Relative fluorescence intensity[a] |
|---|---|---|---|---|
| A | Uninfected | − | 0 | 799 |
| | Uninfected | + | 0 | 1,731 |
| | Infected | + | 84 | 1,857 |
| B | Uninfected | − | 0 | 2,559 |
| | Infected | − | 80 | 2,066 |
| | Uninfected | + | 0 | 3,224 |
| | Infected | + | 70 | 3,553 |

[a] Values given are the mean relative fluorescence intensities of 5,000 cells. Differences in relative fluorescence intensities (mean difference ± standard deviation) were as follows: IFN-γ versus no IFN-γ, 1,028 ± 419; infected versus uninfected, −13 ± 428. Control immunofluorescent staining with mouse myeloma isotypic control IgG2b was negligible (experiment A relative fluorescence intensity, 6.6; experiment B relative fluorescence intensity, 8.8).

ing 50% normal goat serum and 0.1% fish skin gelatin overnight at 4°C, washed with HEPES buffer, and incubated with goat anti-mouse immunoglobulin conjugated to 10 nm colloidal gold for 90 min at room temperature. Sections were washed again with HEPES buffer, postfixed in 4% glutaraldehyde for 5 min, washed in distilled water, and embedded in 2% polyvinyl alcohol containing 0.3% uranyl acetate.

## RESULTS

**Infection with *L. pneumophila* does not alter the overall expression of HLA-DR antigens on human monocytes.** Infection of host cells by parasites has been reported to have variable effects on MHC expression, depending upon the system being studied (3, 4, 13, 27, 31, 32, 34). The effect of *L. pneumophila* infection on MHC expression on isolated mononuclear phagocytes in vitro has not been reported previously. To determine the influence of *L. pneumophila* infection on the expression of HLA-DR antigens on the monocyte surface, we studied infected and uninfected monocytes by two-color flow cytometry in the presence and absence of IFN-γ. As expected, treatment with IFN-γ increased HLA-DR expression on both infected and uninfected monocytes. However, infection with *L. pneumophila* had little effect on HLA-DR expression (Table 1). Within a population of infected monocytes, 70 to 84% were found to be infected with *L. pneumophila* by anti-*L. pneumophila* FITC fluorescence (Table 1). Examination by fluorescence microscopy confirmed this and revealed that the anti-*L. pneumophila* FITC fluorescence was present in a discrete intracellular staining pattern. Two-color flow cytometry analysis revealed that within a population of infected cells, there was no correlation between anti-*L. pneumophila* FITC fluorescence and anti-HLA-DR phycoerythrin fluorescence.

**Paucity of staining for MHC molecules on *L. pneumophila* phagosomes.** To determine the distribution of MHC molecules on phagosomes containing wild-type *L. pneumophila*, we infected monocytes and studied the distribution of MHC class I and class II molecules by the cryosection immunogold technique. We quantitated the intensity of staining by determining the number of gold particles per micrometer of plasma membrane and phagosomal membrane. Whereas staining for HLA-DR, MHC class II invariant chain, and $\beta_2$-microglobulin was abundant on the plasma membrane of infected monocytes (Fig. 1 to 3), staining of these molecules was absent or markedly reduced on *L. pneumophila* phagosomes. HLA-DR staining was absent from the majority (69%) of *L. pneumophila* phagosomes, with staining levels indistinguishable from the background level (Fig. 4). This paucity of staining was observed both on phagosomes in which the *L. pneumophila* had multiplied extensively (Fig. 1) and phagosomes with less-extensive bacterial multiplication (Fig. 2A). On 20% of *L. pneumophila* phagosomes, HLA-DR staining was above the background level but still less than 25% of the intensity of the staining on the plasma membrane, and on 11% of phagosomes, HLA-DR staining was greater than or equal to 25% of the intensity of the staining on the plasma membrane (Fig. 2B and 4). Overall, HLA-DR staining on the phagosomal membrane was 12% of the intensity of the staining on the plasma membrane (Fig. 5). Although staining for MHC molecules was rare on *L. pneumophila* phagosomes, staining for MHC molecules was frequently observed in nonphagosomal cytoplasmic vesicles (Fig. 2B). We obtained similar results when we stained for MHC class II invariant chain, with most *L. pneumophila* phagosomes showing negligible staining and occasional phagosomes showing levels of staining greater than background levels (Fig. 3A and 5). $\beta_2$-Microglobulin staining was consistently absent from *L. pneumophila* phagosomes (Fig. 3B, 4, and 5).

**Paucity of staining for MHC molecules on *L. pneumophila* phagolysosomes.** Whereas wild-type *L. pneumophila* resides in a phagosome that does not fuse with lysosomes, avirulent mutant *L. pneumophila* 25D resides in a phagolysosome. To determine whether the distribution of MHC molecules differs on these two intracellular compartments, we quantitated staining for MHC class I and class II molecules on phagolysosomes containing mutant *L. pneumophila*. Immunogold staining for both $\beta_2$-microglobulin and HLA-DR was consistently negligible on phagolysosomes containing *L. pneumophila* mutant 25D (Fig. 5 and 6).

## DISCUSSION

We have found that infection with *L. pneumophila* in vitro does not alter the overall expression of MHC class II molecules by human mononuclear phagocytes. We have also found that MHC molecules are scarce on the *L. pneumophila* phagosome and do not accumulate on it during phagosome maturation. $\beta_2$-Microglobulin, a marker for MHC class I molecules, is uniformly absent on mature *L. pneumophila* phagosomes, and HLA-DR is absent on two-thirds of phagosomes and present at low levels on one-third.

The basal expression of MHC molecules declines as mononuclear phagocytes are cultured in vitro (33). This decline can be arrested either by the presence of lymphocytes or IFN-γ (33). In vivo, class II MHC expression on rat alveolar macrophages increases after aerosol challenge with *L. pneumophila* or *E. coli* (32), presumably in response to cytokines released as part of an inflammatory response. The effect of *L. pneumophila* infection on MHC expression on isolated mononuclear phagocytes in vitro has not been reported previously. We observed no direct effect of *L. pneumophila* on MHC expression on isolated monocytes in vitro, suggesting that the increase observed in vivo (32) is due to the influence of lymphocytes.

Whereas Antoine et al. (2) observed intense staining for MHC class II antigens on the *Leishmania* parasitophorous vacuole in mouse macrophages, we observed little or no staining for MHC class II molecules on the *L. pneumophila* phagosome in human monocytes. The difference in the

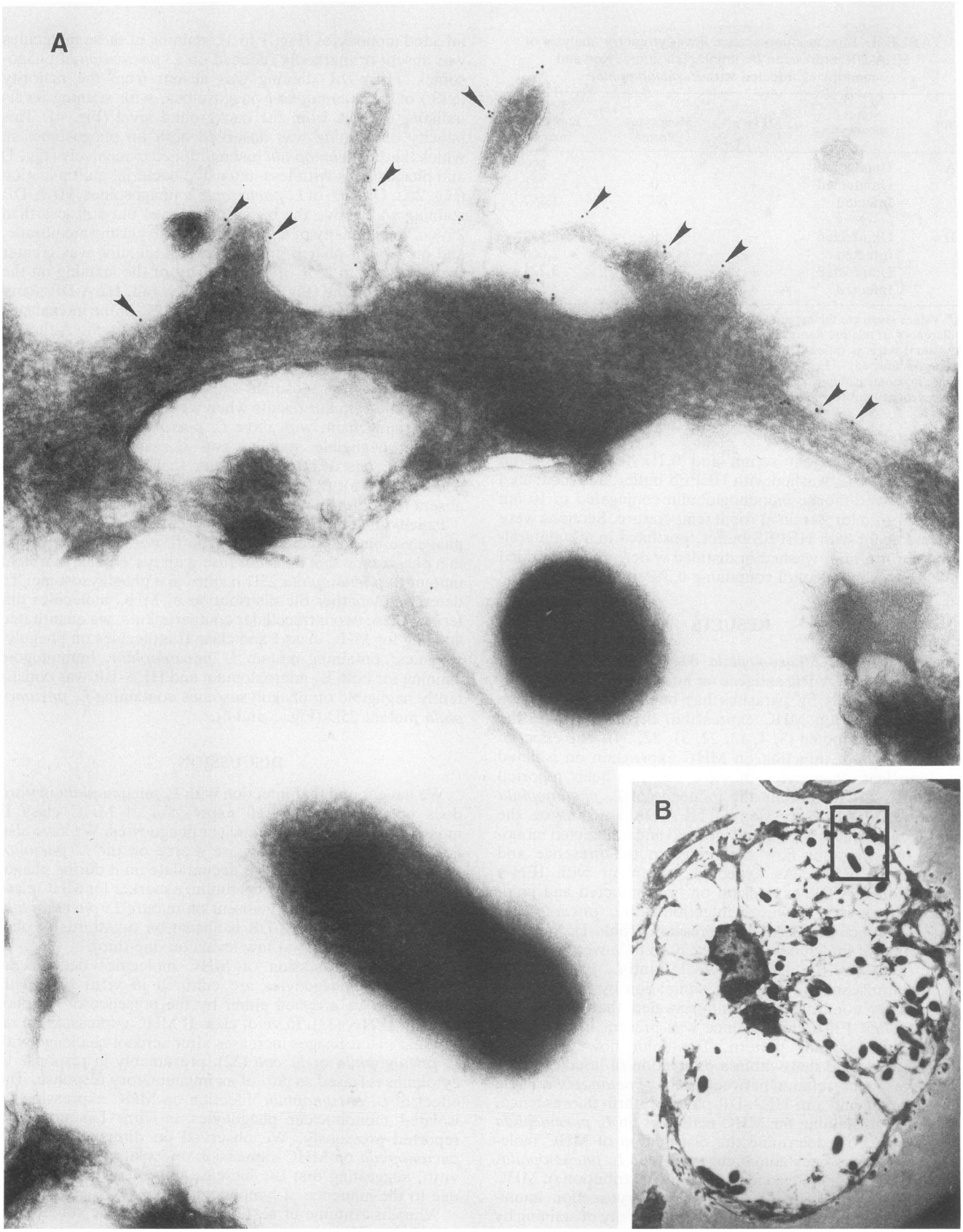


FIG. 1. Cryosection immunogold staining for HLA-DR molecules on a human monocyte with a large *L. pneumophila* phagosome. IFN-γ-treated human monocytes were fixed 22 h after phagocytosis of *L. pneumophila*, and cryosections were stained by immunogold for HLA-DR. Micrograph A is a higher magnification of the area within the rectangle shown in inset micrograph B. Immunogold staining for HLA-DR molecules is abundant on the plasma membrane (arrowheads) but absent from the phagosomal membrane. Magnifications: A, ×65,800; B, ×3,400.

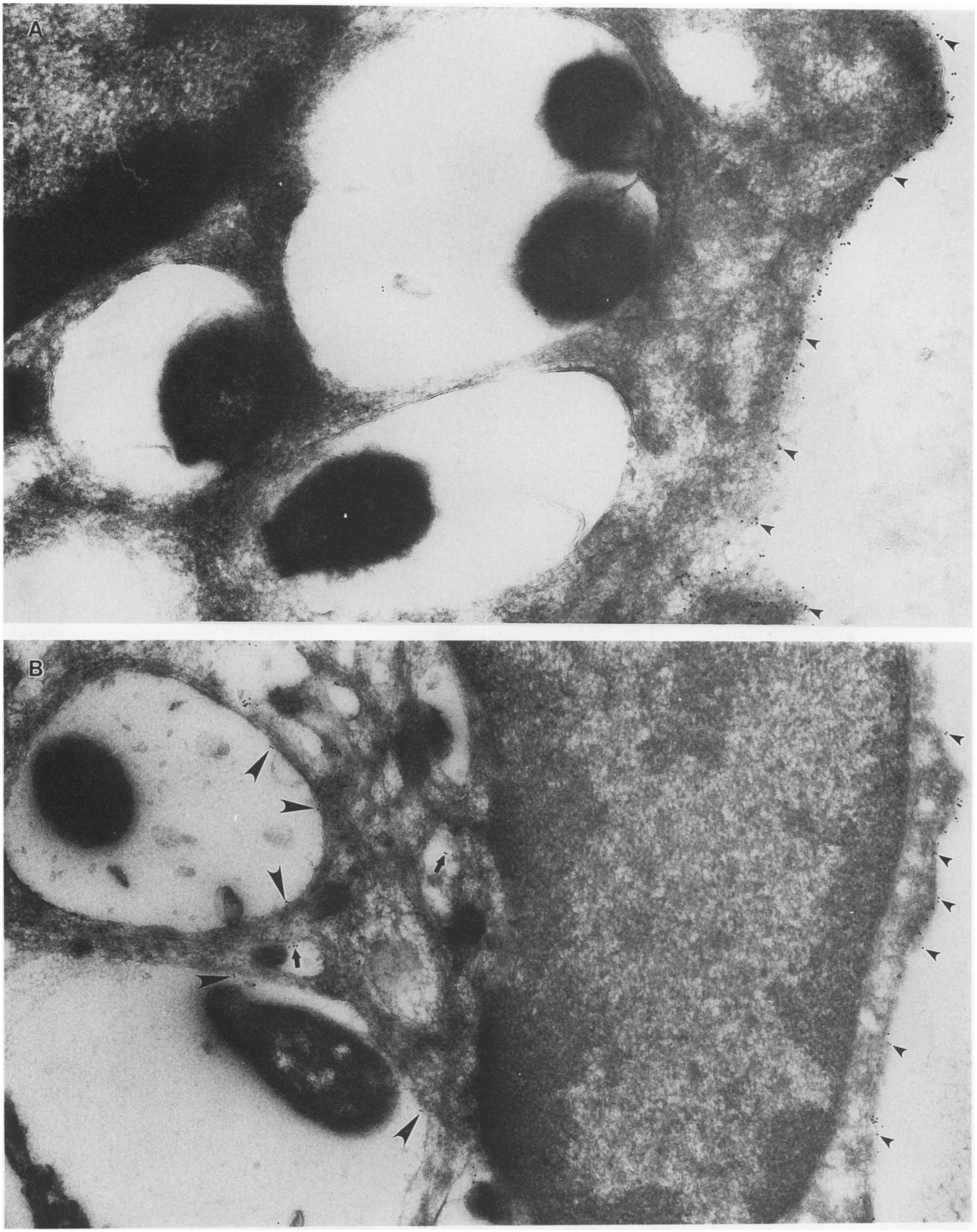


FIG. 2. Cryosection immunogold staining of HLA-DR molecules on monocytes with small *L. pneumophila* phagosomes. IFN-γ-treated monocytes were infected with *L. pneumophila* and stained for HLA-DR as described in the legend to Fig. 1. Immunogold staining is abundant on the plasma membrane (small arrowheads) but is absent (A) or present at low levels (B, large arrowheads) on the phagosomal membrane. Immunogold staining for HLA-DR is frequently observed on cytoplasmic vesicles outside the *L. pneumophila* phagosomes (B, arrows). Magnifications: A, ×52,700; B, ×34,500.

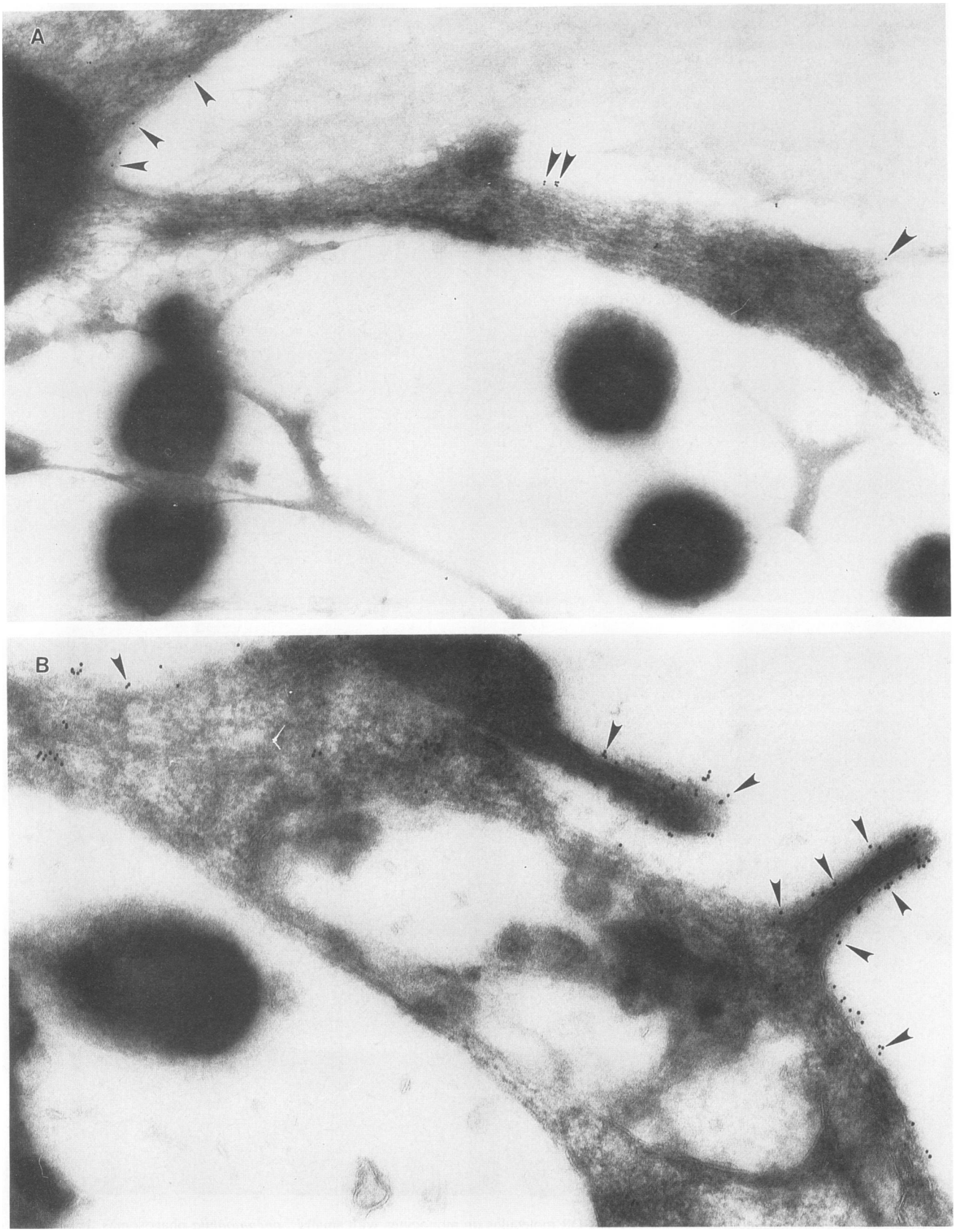


FIG. 3. Cryosection immunogold staining for MHC class II invariant chain and $\beta_2$-microglobulin on *L. pneumophila*-infected monocytes. IFN-γ-treated monocytes were infected with *L. pneumophila* and stained for MHC class II invariant chain (A) or $\beta_2$-microglobulin (B). Immunogold staining for the invariant chain and $\beta_2$-microglobulin is present on the plasma membrane (arrowheads) but absent on the phagosomal membrane. Magnifications: A, ×49,100; B, ×67,900.

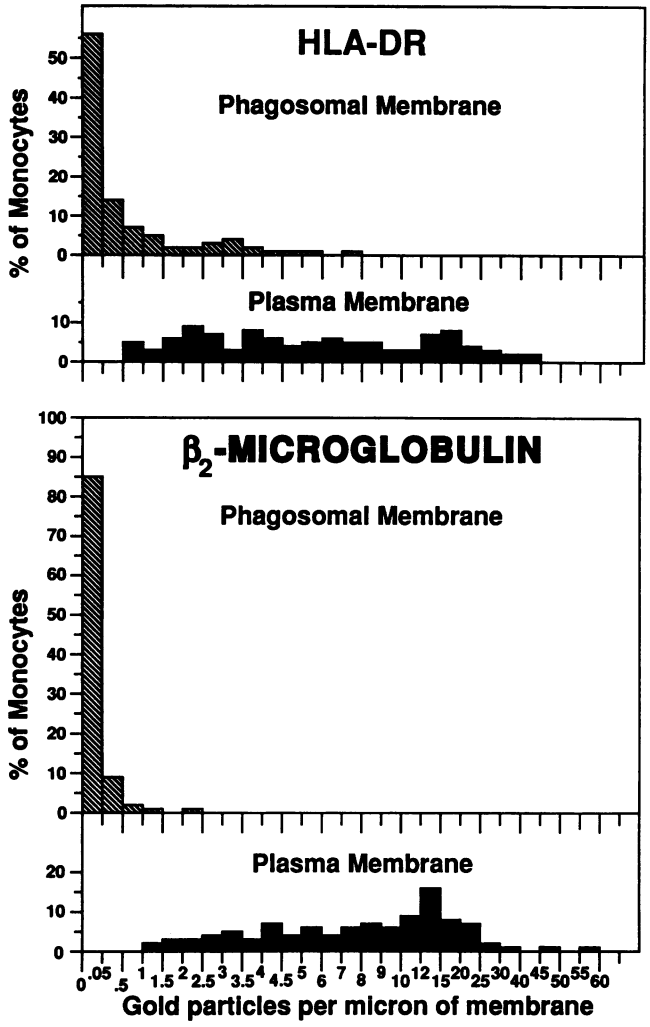


FIG. 4. Distribution of staining intensity for HLA-DR and $\beta_2$-microglobulin on the plasma membrane and the phagosomal membrane of monocytes infected with wild-type *L. pneumophila*. IFN-γ-treated human monocytes were infected with *L. pneumophila* as described in the legend to Fig. 1 and stained by immunogold for HLA-DR or $\beta_2$-microglobulin. The number of gold particles per micrometer of membrane was determined on the plasma membrane and the phagosomal membrane from a total of approximately 120 cells pooled from three different experiments. Immunogold staining with mouse myeloma isotypic control immunoglobulins was negligible (IgG2b, 0.006 ± 0.02 gold particles per μm of plasma membrane and 0.04 ± 0.1 gold particles per μm of phagosomal membrane; IgG1, 0.04 ± 0.02 gold particles per μm of plasma membrane and 0.04 ± 0.02 gold particles per μm of phagosomal membrane). Background levels of staining have not been subtracted from the data shown.

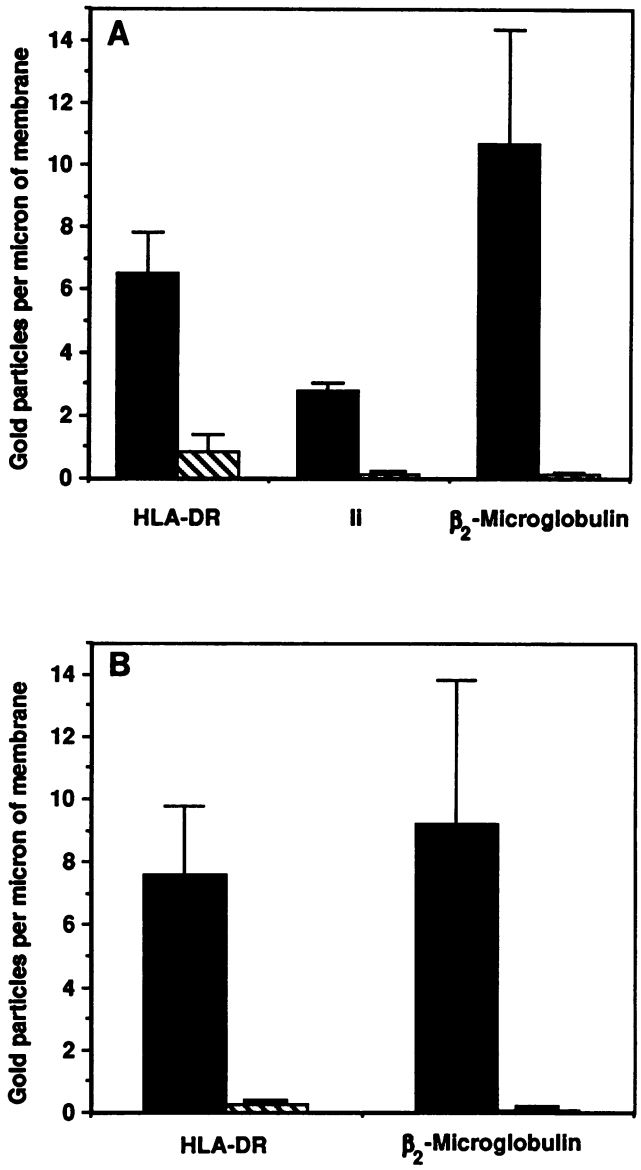


FIG. 5. Hypoexpression of MHC molecules on mature *L. pneumophila* phagosomes and phagolysosomes. IFN-γ-treated human monocytes were infected with wild-type *L. pneumophila* (A) or the avirulent mutant *L. pneumophila* 25D (B) and fixed 22 h later. Cryosections were stained by immunogold for HLA-DR, MHC class II invariant chain (Ii), or $\beta_2$-microglobulin. The number of gold particles per micrometer of membrane was determined on the plasma membrane (■) and the phagosomal membrane (▨). Data represent the mean ± standard deviation of three experiments (A, same cells as those shown in Fig. 4) or the mean ± standard deviation of two experiments (B), with at least 20 cells on each of at least two electron microscopy grids counted in each experiment.

results is not due simply to different methodologies, i.e., cryosection immunogold as opposed to immunoperoxidase staining, since we also observed no staining for MHC molecules in the *L. pneumophila* phagosome by the immunoperoxidase method (unpublished data). The difference in the results is also not due simply to the fact that the *Leishmania* sp. resides in a phagolysosome whereas *L. pneumophila* resides in a phagosome, because HLA-DR staining was also negligible on *L. pneumophila* phagolysosomes containing a mutant bacterium that fails to inhibit phagosome-lysosome fusion. Our finding of minimal HLA-DR immunogold staining in the phagolysosome of the *L. pneumophila* mutant 25D is consistent with evidence that mature lysosomes contain little or no HLA-DR (15, 16). Our finding is also consistent with evidence that phagolysosomes contain few or no MHC molecules. At the light microscope level, Antoine et al. (2) found little or no colocalization of MHC class II immunofluorescence with polystyrene beads

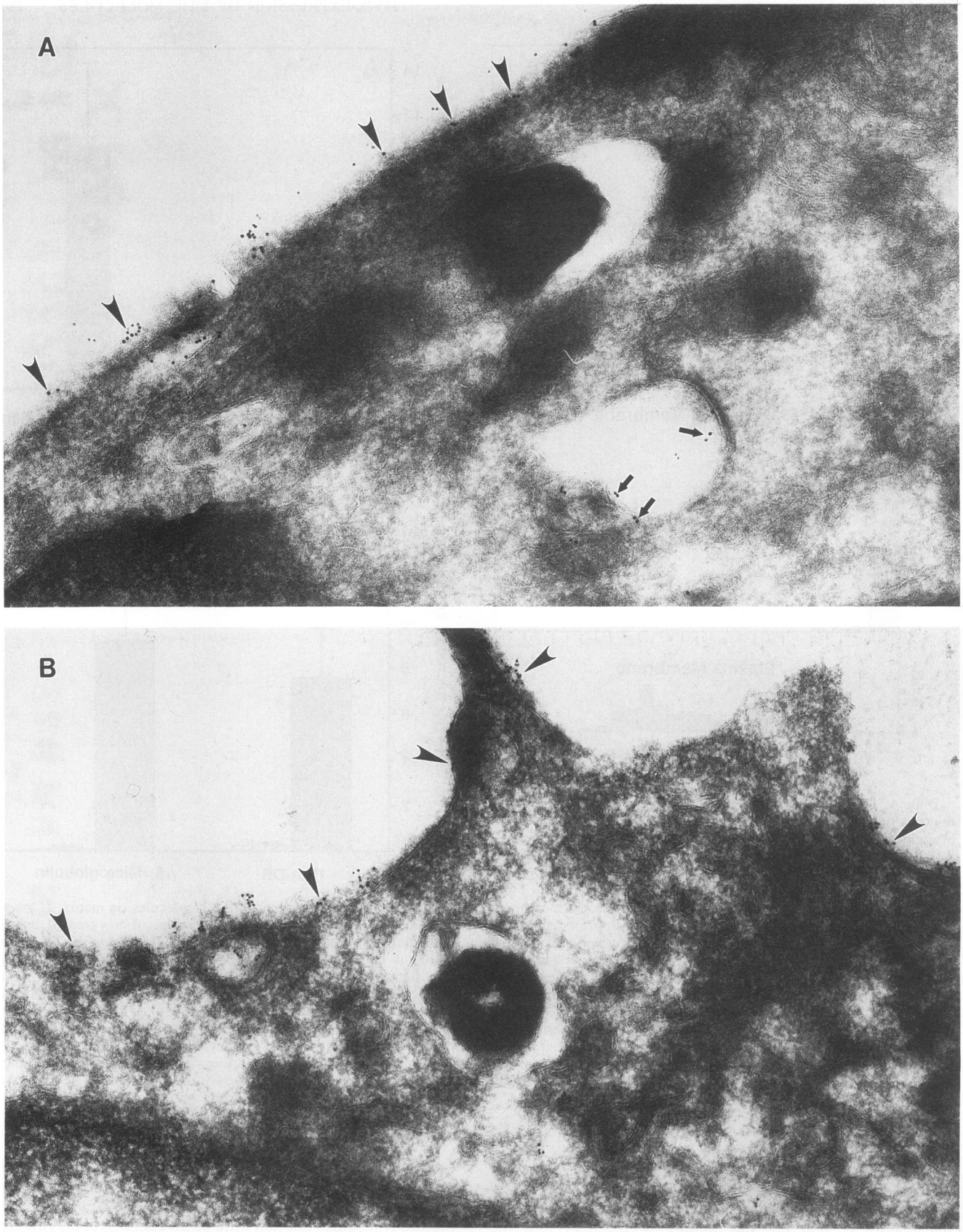


FIG. 6. Cryosection immunogold staining for MHC molecules on monocytes infected with avirulent *L. pneumophila* mutant 25D. IFN-γ-treated human monocytes were fixed 22 h after phagocytosis of *L. pneumophila* mutant 25D. Cryosections were stained for HLA-DR (A) or $\beta_2$-microglobulin (B). Immunogold staining for MHC molecules was abundant on the plasma membrane (arrowheads) and often observed in cytoplasmic vesicles (A, arrows) but absent or rare on the phagosomal membranes. Magnifications: A, ×88,500; B, ×62,900.

ingested by mouse macrophages. At the ultrastructural level, we have found by using the cryosection immunogold technique that IFN-γ-treated human monocytes show intense staining for MHC molecules on the plasma membrane but little or no staining for MHC molecules on phagolysosomes containing latex beads 22 h after phagocytosis (11a).

The high concentration of MHC molecules on the mature *Leishmania* parasitophorous vacuole may reflect a special property of that vacuole. It is also possible, however, that the apparent abundance of MHC class II molecules on the *Leishmania* parasitophorous vacuole was exaggerated by the preembedding immunoperoxidase method. Whereas peroxidase reaction product can diffuse away from the plasma membrane, it may be trapped and relatively intensified within a parasitophorous vacuole. The cryosection immunogold procedure used in our studies is inherently more quantitative and allows a more accurate measurement of the relative abundance of MHC molecules on the phagosomal and plasma membranes.

We have recently found that *Mycobacterium tuberculosis* phagosomes and phagolysosomes in human monocytes, in marked contrast to those of *L. pneumophila*, stain positively for MHC molecules. In the same monocytes, lysosomes labeled with BSA-gold (and then chased overnight) do not stain positively for MHC molecules (11a). These results indicate that parasite intracellular compartments are much more heterogeneous than previously realized. These results also argue against a priori generalizations about the MHC content of parasite phagosomes and underscore the need for careful quantitative measurements in the case of each parasite.

After synthesis, MHC class II molecules, unlike MHC class I molecules, are routed to vesicles in the cell periphery with characteristics consistent with endosomes (28). The transferrin receptor is a marker for endosomes and has been found to colocalize with HLA-DR in an endosomal compartment (14). Consistent with the paucity of staining for MHC molecules in the *L. pneumophila* phagosome, we have also found no immunogold staining for the transferrin receptor in *L. pneumophila* phagosomes (unpublished data), suggesting that there is minimal transfer of endosomal membrane proteins to *L. pneumophila* phagosomes and therefore little accumulation of endosomal membrane proteins in the *L. pneumophila* phagosome. That MHC molecules are scarce in the *L. pneumophila* phagosome suggests that the encounter between *L. pneumophila* T-cell antigens and MHC molecules may take place in an extraphagosomal site within the cell. Alternatively, it is possible that HLA-DR does transit extensively through the *L. pneumophila* phagosome but is rapidly removed so that it cannot accumulate appreciably in the phagosome. Low levels of phagosomal HLA-DR or rapidly transmitting HLA-DR could be important in the processing and presentation of *L. pneumophila* antigens.

Our study demonstrates that the *L. pneumophila* phagosome is fundamentally different from the *Leishmania* vacuole with respect to the accumulation of MHC class II molecules on the phagosomal membrane. It is tempting to speculate that the *Leishmania* parasitophorous vacuole fuses extensively with both endosomes and lysosomes, whereas the *L. pneumophila* phagosome fuses minimally with these organelles. In any case, our study suggests that *L. pneumophila* does not reside in a typical endosomal compartment in the host cell.

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