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Effects of Mycoplasma Contamination on Immunoglobulin Biosynthesis by Human B Lymphoblastoid Cell Lines

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The synthesis and secretion of immunoglobulin M (IgM), as well as the relative ratio of membrane and secretory μ heavy chain (μ_m and μ_s, respectively), were evaluated in mycoplasma-contaminated B lymphoblastoid cell lines. The ratio of μ_m to μ_s was drastically lowered in infected cultures, and μ_s chains could now combine with light chains. A 50 to 100% increase in IgM synthesis occurred in contaminated cultures, and small amounts of IgM were detectable in the culture media. These molecules possessed μ chains typical of secreted IgM. Reexpression of μ on the surface of B lymphoblastoid cells was substantially delayed in mycoplasma-contaminated cultures. Thus, mycoplasma contamination alters the synthesis and expression of a specific differentiated gene product (immunoglobulin) in B cell lines; such changes could significantly affect the interpretation of data on immunoglobulin synthesis by B cells with different phenotypes. This system may also provide a means of studying how mycoplasma infection alters specific gene expression in B cell lines.

The contamination of B lymphoblastoid cell lines with mycoplasmas is a problem shared with other tissue culture systems (8, 15). Some strains of mycoplasma can contaminate cultures without causing gross cellular damage (1), but will drastically alter host cell metabolism (16). Mycoplasmas are also mitogenic for nontransformed human B cells (4, 5, 12) and could potentially alter immunoglobulin synthesis in vitro.

The production of membrane and secretory μ chains (μ_m and μ_s, respectively) in human B cell lines has been well characterized by cell-free synthesis (6, 7, 9, 14, 17) and biosynthetic labeling techniques (17); the ratio of μ_m to μ_s has been found to be ca. 80:20 by both methods. Occasionally in the literature and several times in our own experience, we observed shifts in the ratio of μ_m to μ_s to 50:50 or even 20:80. These variations in the μ_m/μ_s ratio coincided with outbreaks of mycoplasma contamination in our cell lines. In this study, we have investigated (i) the ratio of μ_m to μ_s heavy chains, (ii) their combination with light chains, (iii) the rate of surface immunoglobulin reexpression, and (iv) immunoglobulin M (IgM) secretion in mycoplasma-free and mycoplasma-infected B lymphoblastoid cell cultures. All four parameters were altered by the presence of mycoplasmas.

MATERIALS AND METHODS

Cell lines. Daudi cells, a surface μ^+, κ^+ B cell line derived from a patient with Burkitt's lymphoma (9), F4 cells, a surface μ^+, κ^+ B cell line derived from an individual with hairy cell leukemia, and LBW-2 cells, a surface μ^+ light chain-negative cell line derived from a patient with common variable immunodeficiency (7) were used in this study. The cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml.

Detection of mycoplasmas. The cell lines were routinely screened for the presence of mycoplasmas by staining fixed cytocentrifuge preparations of cells with Hoechst 33528 dye (Sigma Chemical Co., St. Louis, Mo.) (3) and examining the stained cells on a Leitz-Ortho-plan fluorescence microscope equipped with an epi-illuminator. Representative cultures were graciously typed for mycoplasmas by Alan Liss, State University of New York at Binghamton. In all instances, the only species detected was *Mycoplasma hominis*.

Eradication of mycoplasmas. Mycoplasma-infected cell lines were washed and suspended in fresh medium at a density of 10^5 cells per ml. Novobiocin (50 μg/ml; Sigma) was added at the time of suspension and at each feeding. After 1 week, the cells were washed and suspended in medium containing tylosin (60 μg/ml; Gibco Laboratories, Grand Island, N.Y.) and erythromycin (100 μg/ml; Abbott Laboratories, North Chicago, Ill.) and maintained in medium containing these antibiotics for 4 weeks. The cells were then placed in medium without these antibiotics and examined for mycoplasma contamination immediately and after 4 weeks.

Biosynthetic labeling. Cell lines were labeled with [^{35}S]methionine and [^{35}S]cysteine (25 μCi/ml each, >600 Ci/ml; Amersham Radiochemicals, Arlington Heights, Ill.), and immunoglobulin proteins were isolated as described previously (6). Briefly, 2×10^7 labeled cells were lysed with nonionic detergents and immunoprecipitated with either goat anti-human μ or burro anti-human κ (Meloy, Springfield, Va.). Immunoprecipitated material was reduced and separated by electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gels, and labeled proteins were detected by fluorography.

Analysis of fluorograms. X-rays of radiolabeled gels were scanned with a Beckman DU-8 spectrophotometer (Beckman Instrutments, Irvine, Calif.). Areas under the peaks were automatically integrated, and ratios of μ_m and μ_s were calculated from these areas.

Surface regeneration of μ heavy chains. The expression of surface IgM was determined after protease digestion by using a fluorescence-activated cell sorter (FACS IV.5; Becton Dickinson and Co., Mountain View, Calif.) (11). Ten

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thousand cells were counted, and their fluorescence intensity was determined with a logarithmic amplifier. Logarithmic means were calculated and expressed as a percentage of the result for control (undigested) cells stained at the same time.

RESULTS

To determine whether infection with mycoplasma altered the synthesis of μ_m and μ_s, Daudi cells that were either contaminated with mycoplasma or uncontaminated (as determined by Hoechst staining) were radiolabeled with [^{35}S]methionine and [^{35}S]cysteine. The cells were lysed, and IgM was immunoprecipitated with anti-μ serum. Mycoplasma-negative cells produced more μ_m than μ_s, as expected (Fig. 1A); however, mycoplasma-infected cells synthesized greater relative quantities of μ_s than of μ_m (Fig. 1A). Mycoplasma infection did not change the size of either μ_m (78 kilodaltons) or μ_s (74 kilodaltons).

When the ratio of μ_m to μ_s was compared for three cell lines, all demonstrated large reductions in the relative proportions of μ_m versus μ_s after mycoplasma infection (Fig. 2). When quantitative analysis of total IgM synthesis was performed for each cell line, mycoplasma-infected cells synthesized between 50 and 100% more immunoglobulin (per 10^6 cells) than did uninfected cells, as determined by immunoprecipitated radioactivity and integration of areas under both μ chain peaks after densitometric scanning.

Mycoplasma infection did alter the association of light chains with the μ heavy chains. We reported previously that kappa light chains produced in Daudi cells are predominantly associated with the μ_m heavy chains (7). When uninfected Daudi or F4 cells were radiolabeled and lysed and equal portions of the lysate were precipitated with either anti-μ or anti-κ serum, light chains were preferentially associated with μ_m (Fig. 1B; Fig. 3, lane B). When the same procedure was performed on mycoplasma-infected cells, μ_s heavy chains were now associated with kappa light chains (Fig. 1B; Fig. 3, lane B'). Mycoplasma-contaminated F4 cells also secreted IgM with μ chains that possessed a molecular weight greater than that of intracellular μ_s (Fig. 3, lane C), suggesting that such IgM was not merely released from dying cells.

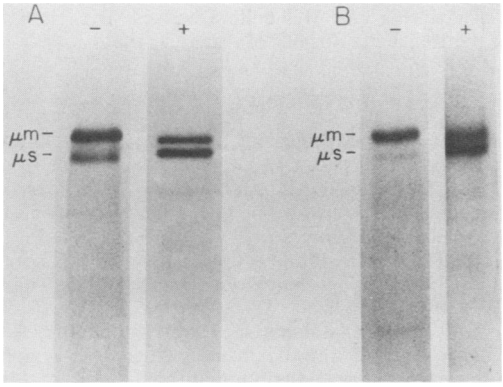

FIG. 1. Immunoprecipitation of μ chains with anti-μ (A) and anti-κ (B) serum in 20 × 10^6 infected Daudi cells labeled with [^{35}S]methionine and [^{35}S]cysteine for 9 h. Immunoglobulin was precipitated from the uninfected (−) and infected (+) cell lysates with either anti-μ or anti-κ, and isolated material was separated by electrophoresis under reducing conditions.

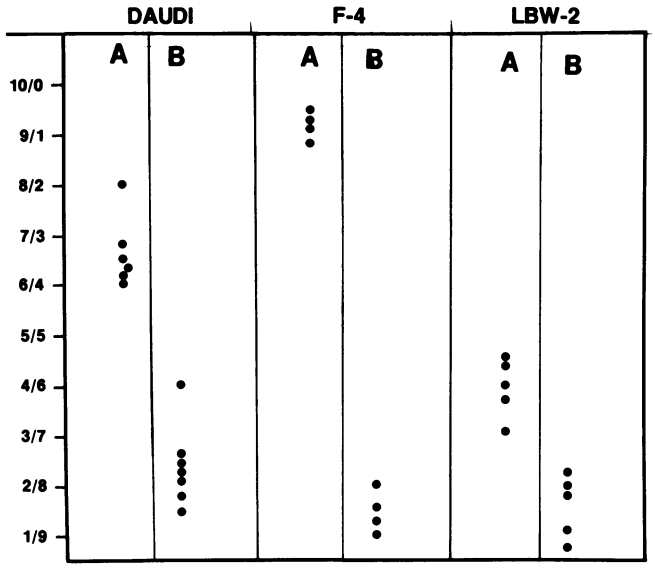

FIG. 2. Analysis of μ_m/μ_s ratios in control (A) and mycoplasma-infected (B) B cell lines. The μ chain bands on fluorograms of sodium dodecyl sulfate gels were scanned with densitometer, and the areas under μ peaks were automatically integrated. The μ_m/μ_s ratio is shown on the left-hand scale. Each data point represents a single determination.

The rate of reexpression of surface IgM after protease digestion was greatly retarded in mycoplasma-infected cells (Fig. 4). When uninfected Daudi and LBW-2 cells were examined, 50% of the surface IgM was reexpressed within 6 h, and complete regeneration occurred within 15 h after protease treatment (Fig. 4). However, in mycoplasma-infected cells, only 50% of the surface IgM was regenerated in Daudi cells after 18 h (24 h for LBW-2 cells), and complete regeneration did not occur until 24 h after protease treatment (33 h for LBW-2 cells) (Fig. 4). In addition to a decrease in the rate of reexpression, the amounts of surface IgM expressed on these cell lines were diminished fourfold during mycoplasma infection as determined by quantitative surface enzyme-linked immunosorbent assay and by fluorescence-activated cell sorter analysis of fluorescence intensity (data not shown).

No alteration in cell proliferation could be detected in mycoplasma-infected cultures. Both infected and uninfected Daudi and F4 cells cultures demonstrated a mean doubling time of 21 h and maintained greater than 95% viability as determined by exclusion of 1% trypan blue dye.

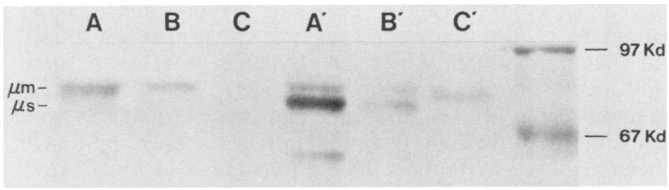

FIG. 3. Synthesis and secretion of IgM by 20 × 10^6 uninfected (lanes A, B, and C) and mycoplasma-infected (lanes A', B', and C') F4 cells labeled with [^{35}S]methionine for 16 h. IgM was precipitated from cell lysates with anti-μ (lanes A and A') or anti-κ (lanes B and B') serum or from the culture medium with anti-μ (lanes C and C') and then separated by electrophoresis under reducing conditions. Fragment sizes (in kilodaltons) are shown.

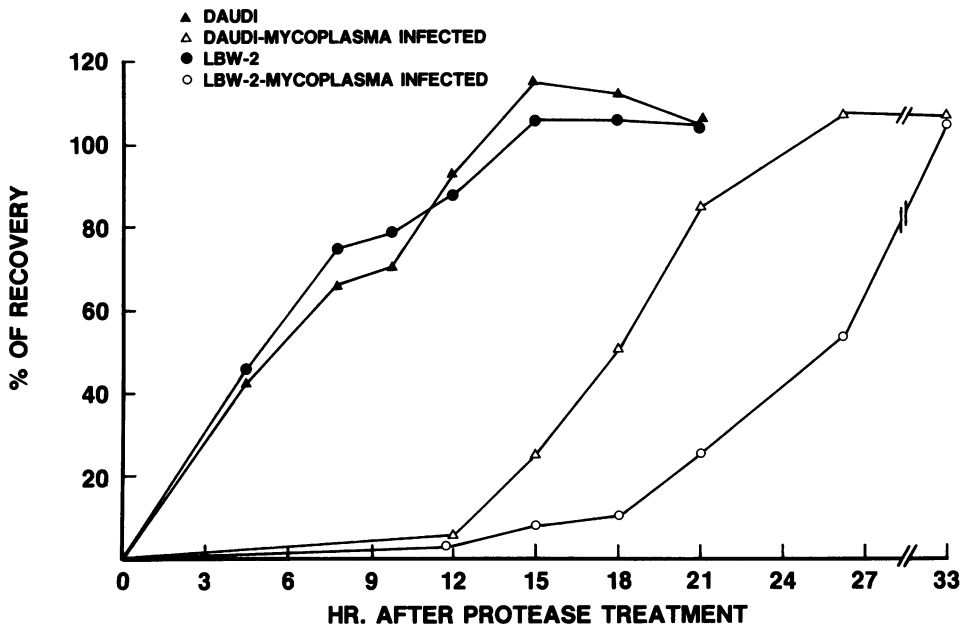

FIG. 4. Regeneration of surface μ in uninfected and mycoplasma-infected cells treated with 1.5 mg of protease per ml for 30 min at 37°C to remove surface proteins and then washed and recultured. Cells were harvested and surface stained with fluorescein isothiocyanate-conjugated anti-human μ serum at the indicated times. Control (undigested) cells were also stained at each time point. Both control and protease-treated cells were then analyzed with a fluorescence-activated cell sorter, and logarithmic means of intensity were calculated. The fluorescence in protease-treated cells was expressed as a percentage of that in the control cells for each time point.

DISCUSSION

Mycoplasma contamination is a common problem in laboratories that use continuously growing cell lines. However, the effect of mycoplasma contamination on specific differentiated cell functions vis à vis cell proliferation has been little studied (2, 10). The data presented here show that mycoplasma infection of human B cell lines causes significant perturbation of immunoglobulin synthesis, processing, and expression. The ratio of μ_m to μ_s, the rate of reexpression of μ_m on the cell surface, and the total amount of surface μ_m in Daudi cells were all acutely diminished, whereas the synthesis and secretion of IgM were enhanced. Such effects of mycoplasma infection could alter the results and interpretation of studies examining the expression of membrane and secretory IgM, especially when only small amounts of μ_s are normally present on cells (e.g., B lymphocytes treated with tunicamycin and certain pre-B-like cell lines). Some inconsistent data for immunoglobulin synthesis and alterations of B cell line phenotypes might be explained by periodic mycoplasma infection of long-term cultures.

The increased production of μ_s and its association with light chains in Daudi and F4 cells after mycoplasma infection suggest a change in μ chain synthesis and processing similar to that normally observed in plasma cells (11). In some instances (Fig. 3), IgM molecules were secreted in small amounts into the culture medium, which indicates that capabilities other than the production of secretory IgM have been acquired by these cells (e.g., synthesis of J chains).

It is unclear whether the results of mycoplasma infection observed here are due to the interaction of mycoplasmas with a specific mitogenic receptor on B cell lines (4, 5, 12), perturbation of the cell surface membrane during the membrane-associated phase of the mycoplasma growth cycle (1), or alternation of medium composition and disruption of normal cell metabolism (13). However, laboratories that use B lymphoblastoid cell lines to examine the regulation of immunoglobulin synthesis must regularly check their cell lines for mycoplasma contamination and use only uninfected cells; otherwise, discrepant results and interpretative errors may distort their findings. Finally, these studies also provide a useful, reproducible system for analyzing the interactions between specific mycoplasma-produced molecules and B lymphoblastoid cells by measuring their effects on specialized gene products.

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