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Lack of Effect of Mouse Adenovirus Type 1 Infection on Cell Surface Expression of Major Histocompatibility Complex Class I Antigens

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It has been proposed that adenoviruses establish and maintain persistent infections by reducing the class I major histocompatibility complex-associated presentation of viral antigens to cytotoxic T lymphocytes, leading to ineffective cell-mediated immunity and impaired clearance of infected cells (W. S. M. Wold and L. R. Gooding, Virology 184:1–8, 1991). Early region 3 of human adenovirus types 2 and 5 encodes a 19-kDa glycoprotein that associates with the class I major histocompatibility complex (MHC) antigens in the endoplasmic reticulum and prevents their maturation and transport to the cell surface. Early region 1A of human adenovirus type 12 encodes a protein that inhibits class I MHC mRNA production at the transcriptional or posttranscriptional processing level. Unlike human adenovirus infections, however, mouse adenovirus type 1 (MAV-1) infection of a variety of cell types did not affect the surface expression of 10 different mouse class I MHC allotypes. MAV-1-infected cells also regenerated cell surface class I MHC antigens following proteolytic removal as efficiently as mock-infected cells. The ability of cells to present antigen to class I MHC (K^b)-ovalbumin-specific T-cell hybridoma cells was likewise unaltered by MAV-1 infection. Thus, the ability of MAV-1 to persist cannot be explained by the model of reduced class I MHC-associated antigen presentation proposed for human adenoviruses.

The importance of cytotoxic-T-lymphocyte (CTL)-mediated immune responses in limiting and clearing viral infections has been well documented in a number of viral systems (13, 51). Class I major histocompatibility complex (MHC) antigens of infected cells present viral antigens to CTLs, resulting in CTL-mediated lysis and clearance of those cells. Interference with T-cell-mediated immune surveillance is thought to allow viral escape from immune clearance as well as persistence in the host (6, 12, 48, 50).

Human adenoviruses have at least two mechanisms for reducing the surface expression of the class I MHC antigens. Early region 3 (E3) of human adenovirus types 2 and 5 (Ad2/5) encodes a 19-kDa glycoprotein (E3 gp19K) that associates with the class I MHC antigen within the endoplasmic reticulum (ER) (1, 7, 28, 32, 42). The E3 gp19K-class I MHC antigen complex is anchored in the ER by the E3 gp19K ER retention signal, which prevents the maturation and cell surface expression of the anchored class I antigens (10). E1 of Ad12 encodes a protein (E1A) that inhibits class I MHC mRNA production at transcriptional or posttranscriptional processing steps (40). In addition, Ad12-transformed cells have decreased expression of the peptide transporter genes *TAP1* and *TAP2* (33), whose encoded proteins transport processed peptides to the ER for binding to class I MHC molecules (25, 30, 46). It has been proposed that adenoviral persistence is due to down-regulation of class I MHC antigen expression on the surfaces of host cells (23, 48).

While it is clear that human adenovirus infection results in a reduction in cell surface levels of class I MHC antigens in at least some cell types, Routes and Cook (35) have shown that a majority of Ad2/5-infected human cells do not show major decreases in cell surface levels of class I MHC antigen until very late times postinfection, when cell death is imminent. The one notable exception to this is the Ad5 E1-transformed cell line 293. In 293 cells the level of decreased surface expression of class I MHC antigens caused by Ad5 infection correlates with the level of endogenous E1A expression (35, 36).

E3 gp19K has different binding affinities for, and differentially affects the surface expression of, various mouse class I MHC antigens (9, 39, 43). The effects of class I MHC cell surface level reduction on cell-mediated immune responses to infection and on adenoviral persistence have not been directly demonstrated. In Ad2/5 infection of cotton rats, E3 gp19K is involved in limiting the lymphocyte and monocyte/macrophage response and the extent of pneumonia but the effect of E3 gp19K expression on viral persistence has not been analyzed (16). Recombinant vaccinia viruses that express the Ad2 or Ad5 E3 gp19K protein do not show any significant differences in viral replication, lethality, or effect on CTL and natural killer cell responses in mice (11, 17).

The study of mouse adenovirus type 1 (MAV-1) provides a useful model of host-virus interactions in which both virus and host can be manipulated. MAV-1 infection of immunocompetent mice is most often associated with a subclinical, persistent infection (31). Persistence is manifested by high serum antibody titers (19, 47) and prolonged viruria (up to 24 months postinfection) in the absence of overt clinical signs (18, 47). Thus, mouse adenovirus can be used to assess the biological importance of the interactions between adenoviral and host-cellular proteins in the natural host.

We analyzed the surface expression of class I MHC antigens and the class I MHC-associated antigen presentation of MAV-1-infected cultured mouse cells. The results of these experiments indicated that MAV-1 infection did not alter mouse class I MHC cell surface expression or antigen presentation. The implications of these results for the molecular basis of persistence in MAV-1-infected animals and for human adenovirus infections are discussed.

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MATERIALS AND METHODS

Cells. L929 cells were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented

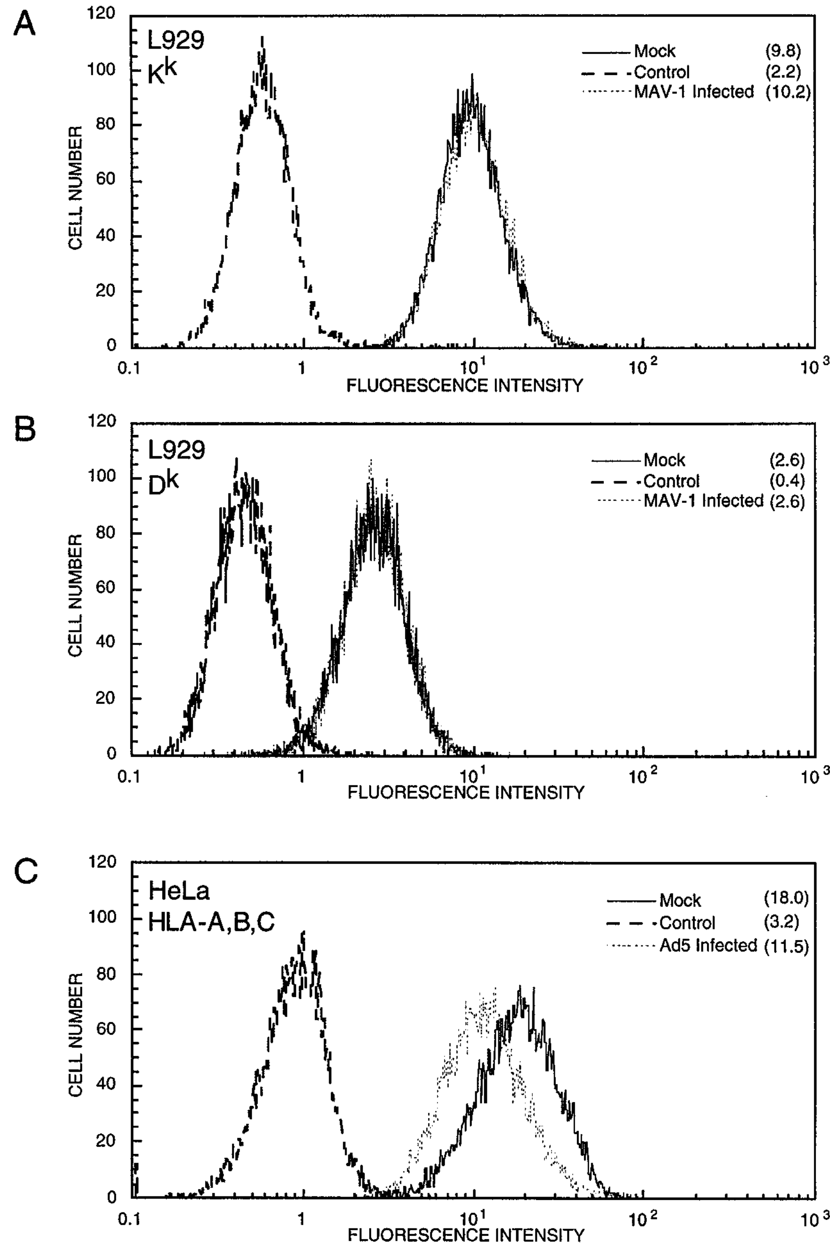

FIG. 1. Flow cytometry analysis of class I MHC antigen expression on the surface of adenovirus-infected cells. (A and B) L929 cells either mock infected or infected with MAV-1 at a multiplicity of 5 PFU per cell for 36 h were labeled with FITC-conjugated anti-H-2K^k (A) or anti-H-2D^k (B) antibodies. Negative control cells were treated with anti-H-2L^d antibody and then labeled with FITC-conjugated anti-mouse IgG antibody. (C) HeLa cells either mock infected or infected with Ad5 at a multiplicity of 5 PFU per cell for 18 h were labeled with FITC-conjugated anti-human HLA-A,B,C antibody. Negative control cells were labeled with FITC-conjugated anti-mouse IgG antibody. The mean fluorescence intensity for each curve is given in parentheses beside the key to each curve.

with 5% heat-inactivated bovine calf serum (Gibco/BRL). HeLa and 293 cells (American Type Culture Collection) were cultured in DMEM supplemented with 7 and 10% newborn bovine serum, respectively (Hazleton Biologics, Inc.). SVB6KH, SSSV, and CQ15 cell lines were obtained from Linda Gooding (Emory University) and were maintained in DMEM with 8% fetal bovine serum (FBS) (Gibco/BRL). RAG-1 cells (American Type Culture Collection) were cultured in Eagle's modified media supplemented with Hanks balanced salt solution, twice the concentration of amino acids and vitamins, and 10% FBS. 37.1 cells, which are 3T6-derived cells that express the MAV-1 E1A gene region under control of the mouse mammary tumor virus promoter (41), were cultured in DMEM with 5% heat-inactivated bovine calf serum and 200 μg of G418 (Gibco/BRL) per ml. E1A expression was induced in 37.1 cells by addition of 10^{-5} M dexamethasone (Sigma Chemical Co.) to the culture media. C57SV cells (29) were obtained from Barbara Knowles (Jackson Laboratory) and cultured in DMEM plus 10% FBS. Rick Tarleton (University of Georgia) supplied the interleukin-2 (IL-2)-producing ovalbumin-specific T-cell hybridoma cell line (B3.6.4.5) (26), which was cultured in DMEM with 10% FBS and 400 μg of G418 per ml, and cultured T-cell growth factor-dependent CTLL-2 cells (44). All cells were cultivated in a 5% CO_2 atmosphere.

Viruses and antibodies. MAV-1 was obtained and passaged as previously described (3), and titers were determined on L929 cells. Ad5 was obtained from Arnold Berk (University of California, Los Angeles) and passaged in HeLa cells; titers were determined on 293 cells. Virus was adsorbed onto monolayers of cells at a multiplicity of 5 or 100 PFU per cell for 1 h at 37°C. Virus was removed, and cells were rinsed in phosphate-buffered saline (PBS) and cultured for an additional 22, 36, or 44 h in DMEM supplemented with 2% of the appropriate serum. For each experiment additional plates of mock- and MAV-1-infected cells were prepared and the success of the infection was monitored by the appearance of cytopathic effect.

Mouse anti-mouse monoclonal antibodies made against the following H-2

TABLE 1. Class I MHC antigens examined

Cell line[a]	Antigen(s)
L929	K^k, D^k
SVB6KH	K^b, D^b
CQ15	K^q, D^q
RAG-1	K^d, D^d, L^d
SSSV	D^s
37.1	K^q, D^q

[a] Cell lines were able to support the replication of MAV-1, defined as a minimum of a sixfold amplification of virus (assayed on L929 cells) following 4 days of incubation.

antigens were purchased from Pharmingen: D^k, K^k, D^b, K^b, D^d, L^d (which cross-reacts with D^b and D^q), K^q, K^s, and D^s (which cross-reacts with K^d). The anti-K^k and -D^k antibodies were purchased as fluorescein isothiocyanate (FITC) conjugates. FITC-conjugated goat $F(ab')_2$ anti-mouse immunoglobulin G (IgG) (Fisher Scientific) was used as a secondary antibody for visualization of the non-FITC-conjugated antibodies for flow cytometry and used alone as a negative control for staining. FITC-conjugated mouse anti-human HLA-A,B,C antibody (Pharmingen) was used for HeLa cell analysis.

Immunofluorescent labeling and analysis. Cells were rinsed with PBS, trypsinized, and washed in PAB (PBS supplemented with 0.02% sodium azide and 1% bovine serum albumin). A total of 10^6 cells were incubated 30 min on ice in 50 μl of PAB with 1 μg of primary antibody per μl. Cells were subsequently washed twice in PAB. When necessary, labeling with a secondary antibody was performed identically to the primary labeling. After washing, cells were resuspended to a final concentration of 10^6/ml of PAB. Intensity of surface staining was detected by flow cytometry (Epics Elite; Coulter). Dead cells were detected by staining with 50 μg of propidium iodide (Sigma Chemical Co.) per ml and were gated out of the analysis. Relative differences in fluorescence intensities were calculated by subtracting the mean fluorescence intensity of unstained cells from that of the labeled cells and then comparing the relative intensities of the labeled cells.

BFA and papain treatment. MAV-1-infected or mock-infected cell monolayers were exposed to 5 μg of brefeldin A (BFA) (Sigma Chemical Co.) per ml for 1 to 3 h and then subjected to immunofluorescent labeling and analysis.

Papain treatment was performed essentially as previously described (38). Briefly, MAV-1-infected or mock-infected cell monolayers were trypsinized, washed in 0.15 M NaCl–0.01 M cysteine–0.01 M Tris-Cl (pH 7.5), resuspended at 1.5×10^7 cells per ml in the same buffer in the presence or absence of 10 mg

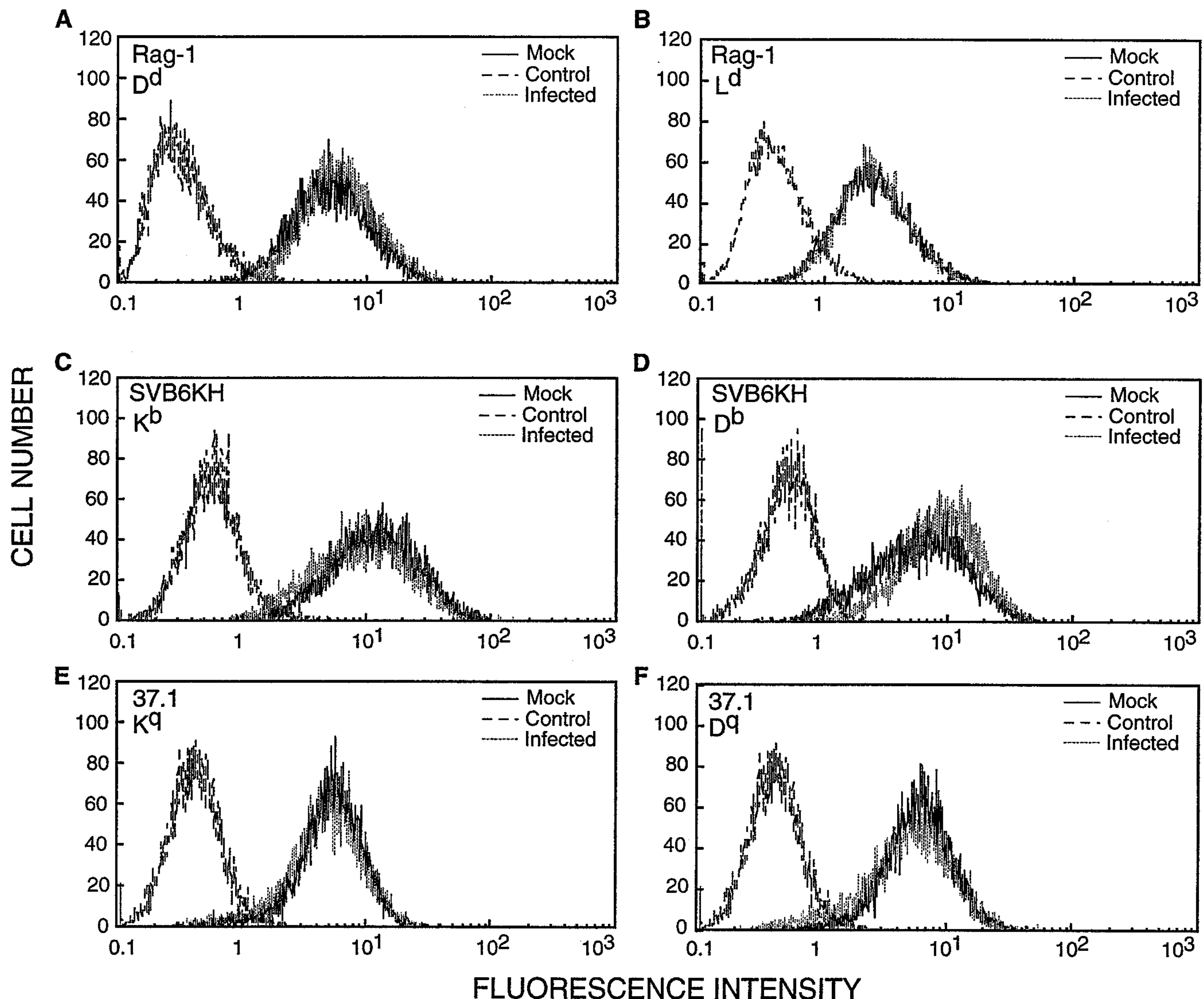

FIG. 2. Flow cytometry analysis of cell surface expression of class I MHC antigens on MAV-1-infected cells. MAV-1 was used at a multiplicity of 5 PFU per cell for 36 h. Mock-infected and infected RAG-1 cells (A and B), SVB6KH cells (C and D), and 37.1 cells (E and F) were treated with primary antibodies against the following H-2 antigens: D^d (A), L^d (B), K^b (C), D^b (D), K^q (E), and D^q (F). They were then labeled with FITC-conjugated anti-mouse IgG antibody. Negative control cells were treated with primary antibodies against H-2 antigens not expressed on their surfaces, as follows: K^b (A, E, and F), K^s (B and D), and D^d (C). They were then labeled with FITC-conjugated anti-mouse IgG antibody.

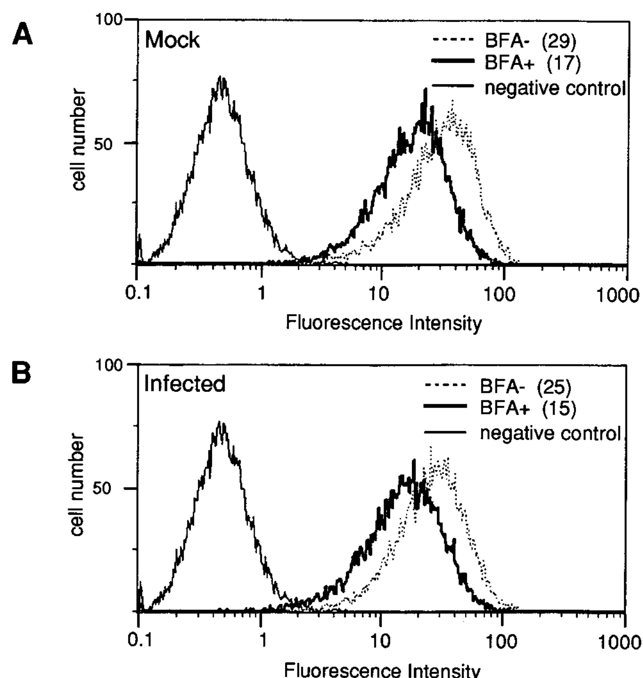

FIG. 3. Flow cytometry analysis of class I MHC surface expression on cells treated with BFA. SVB6KH cells were mock infected (A) or infected with 5 PFU of MAV-1 per cell (B) for 36 h. Cells were treated with BFA for an additional 3 h (BFA+). Control cells were not treated (BFA−). Cells were incubated with anti-H-2K^b antibody and then labeled with FITC-conjugated anti-mouse IgG. Negative control cells were labeled with FITC-conjugated anti-H-2K^k antibody. The mean fluorescence intensity minus the fluorescence intensity of the negative control cells is indicated in parentheses beside the key for each curve.

of crude papain (Sigma Chemical Co.) per ml, and incubated in suspension for 1 h at 37°C. Cells were subsequently resuspended in PAB and held on ice or incubated in growth media for 0.5 to 3 h to allow regeneration of surface antigen. Class I MHC surface antigen levels on control or papain-treated mock-infected or infected cells were analyzed by immunofluorescent labeling and analysis.

IL-2 assay. Antigen-presenting cells (C57SV and SVB6KH, both K^b) were plated at a density of 5 × 10^6/ml and MAV-1 or mock infected. At 12 h postinfection, 1 μg of ovalbumin peptide (gift from Rick Tarleton) (34) per ml was added. At 24 h postinfection 10^5 cells of the IL-2-producing, T-cell hybridoma cell line B3.6.4.5 (specific for the K^b-presented ovalbumin peptide) (22) per ml was added to the antigen-presenting cells and incubated for an additional 16 h. At 40 h postinfection, culture supernatants were collected and stored at −20°C. IL-2 activity was analyzed by quantitation of the 6-h [^{3}H]thymidine incorporation of IL-2-dependent CTLL-2 cells cultured for 18 h in the presence of serial twofold dilutions of the culture supernatants (15, 45).

RESULTS

The surface expression of H-2K^k or H-2D^k antigens on L cells is not altered by MAV-1 infection. To determine whether MAV-1 infection alters the surface expression of class I MHC antigens, cultured cells were infected with MAV-1 and the level of class I MHC antigens presented at the cell surface was detected by immunofluorescent staining. L929 cells were infected with 5 PFU of MAV-1 per cell or mock infected for 22, 36, or 40 h. The cells were labeled with FITC-conjugated antibodies to either the K^k or the D^k class I MHC antigens, and fluorescence intensity was quantitated by flow cytometry. No alteration of surface expression of either the H-2K^k or the H-2D^k antigens was detected on MAV-1-infected cells compared with expression on mock-infected cells (Fig. 1A and B).

Previous reports demonstrating the ability of Ad2/5 to diminish class I MHC surface expression have used a multiplicity of at least 100 PFU per cell (1, 20, 35, 36). The titers of our MAV-1 stocks permitted a multiplicity of only 5 PFU per cell. To demonstrate that the lack of an observable effect of MAV-1 infection on class I MHC surface expression was not a result of the lower multiplicity of infection used, we measured the class I MHC surface expression on HeLa cells infected with Ad5 for 12 and 18 h at a multiplicity of 5 PFU per cell. This low-multiplicity Ad5 infection was capable of effecting a reduction in the surface expression of class I MHC antigens compared with expression on mock-infected cells (Fig. 1C), indicating that Ad5 down-regulation of class I MHC surface expression does not require a high-multiplicity infection. The reduction in cell surface expression at 18 h postinfection shown in Fig. 1C was 44%. Comparable values were seen in duplicate experiments.

Cell surface expression of many different class I MHC antigens is not altered by MAV-1 infection. The ability of the Ad2/5 E3 gp19K protein to bind and inhibit the surface expression of class I MHC antigens varies with the class I MHC alleles expressed. The Ad2 gp19K associates with the mouse H-2K^d and D^b but not K^b or K^k antigens (8, 9, 43). To determine whether MAV-1 is able to alter surface expression of class I MHC antigens other than those shown in Fig. 1, we obtained additional cultured cell lines expressing different *H-2* alleles. Table 1 lists cell lines that supported productive MAV-1 infections and the class I MHC antigens analyzed with each cell line. Each class I MHC haplotype antigen was analyzed individually to determine if MAV-1 infection altered its surface expression. None of the class I MHC antigens expressed by the 10 different *H-2* alleles tested (*H-2K^b*, *H-2K^d*, *H-2K^k*, *H-2K^q*, *H-2D^b*, *H-2D^d*, *H-2D^k*, *H-2D^q*, *H-2D^s*, and *H-2L^d*) displayed reduced surface expression following infection of the cells with MAV-1 (Fig. 2A through D and data not shown). Duplicate experiments gave identical results.

Endogenous expression of MAV-1 E1A does not enable MAV-1 infection to alter class I MHC surface expression. Routes et al. (35, 36) demonstrated a correlation between the level of endogenous E1A expression and the degree to which Ad5 infection decreases the levels of class I MHC cell surface antigens. We therefore analyzed whether increased levels of MAV-1 E1A provided by endogenous expression would affect class I MHC surface expression in MAV-1-infected cells. We have generated a cell line (37.1) in which MAV-1 E1A expression can be induced to levels similar to those detected at early times during a viral infection (41, 49). MAV-1-infected 37.1 cells have enhanced levels of E1A expression compared with infected cells of the parent cell line, 3T6 (49). E1A expression was induced in 37.1 cells for 24 h prior to and throughout the 22- or 36-h infection period. No difference in the surface levels of H-2K^q or H-2D^q antigens between mock- and MAV-1-infected 37.1 cells was detected (Fig. 2E and F).

MHC class I surface antigen levels are decreased by inhibition of cellular transport. To demonstrate that inhibition of cellular transport would result in a detectable decrease in surface class I MHC antigen levels on the mouse cell lines used in the experiments described above, we used an inhibitor of protein secretion and membrane transport, BFA. Treatment with BFA blocks membrane transport out of the ER by redistributing *cis* and medial Golgi components back to the ER (24). SVB6KH cells (*H-2K^b*) were MAV-1 or mock infected for 20 or 36 h prior to treatment with BFA for 3 h. MAV-1-infected and mock-infected cells not treated with BFA served as controls. Treatment with BFA caused a decrease in H-2K^b surface expression of both mock- and MAV-1-infected cells to exactly the same degree: 56% of that of untreated cells (Fig. 3). This demonstrated that inhibition of cellular transport of the class I

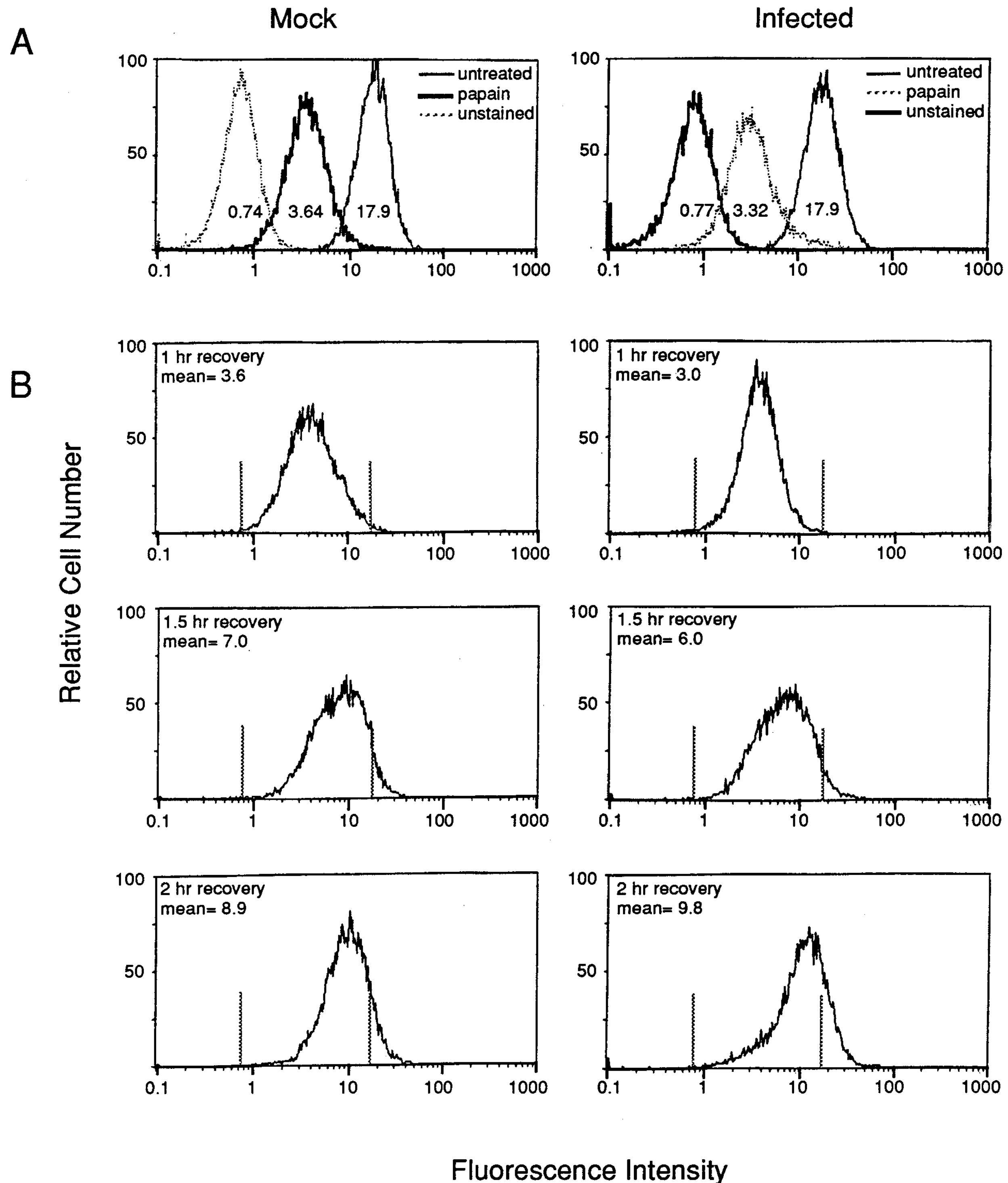

FIG. 4. Removal and regeneration of surface class I MHC antigens on mock- and MAV-1-infected cells treated with papain. (A) L929 cells were mock infected or infected with 5 PFU of MAV-1 per cell for 22 h and treated with 10 mg of papain per ml or left untreated. Treated and untreated cells were labeled with FITC-conjugated anti-H2-K^k antibody. A sample of untreated cells was labeled with FITC-conjugated anti-mouse IgG as a negative control. The intensity of staining was analyzed by flow cytometry. The mean fluorescence intensity for each treatment is indicated beneath each curve. (B) Regeneration of surface antigens was analyzed on cells that were treated with papain as for panel A and allowed to recover in culture media for the indicated times. Vertical bars indicate the peak positions of the unstained or untreated controls in panel A. The mean equals the mean fluorescence intensity minus the mean intensity of the unstained cells.

MHC antigens resulted in a clearly detectable decrease in cell surface antigen levels.

Cell surface expression of newly synthesized class I MHC antigens is not prevented by MAV-1 infection. To examine the effect of MAV-1 infection on newly synthesized class I MHC antigens, cell surface antigens were removed by proteolytic treatment with papain, and regeneration of surface antigen expression was observed following various times of recovery.

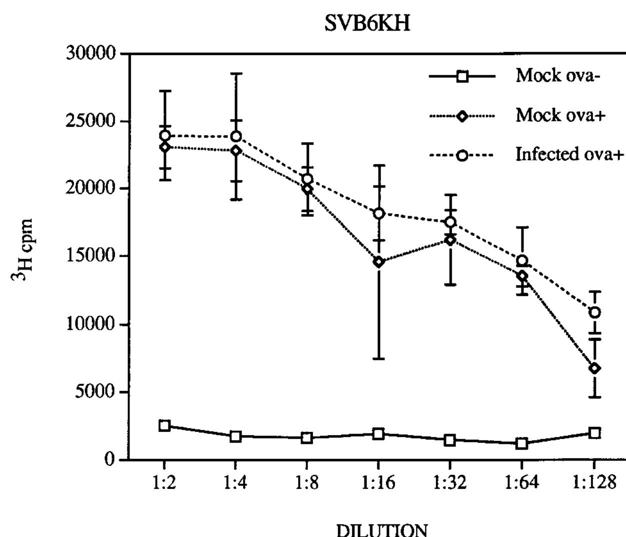

FIG. 5. Mock- and MAV-1-infected cells present exogenously added antigen to T-cell hybridomas equally. Mock- and MAV-1-infected SVB6KH (K^b) cells were analyzed for their ability to present exogenously added ovalbumin (ova) peptide to IL-2-secreting T-cell hybridoma cells specific for peptide presented in the context of the K^b antigen. Mock-infected cells in the absence of peptide were used as negative controls. The ability of serial dilutions of the culture medium (dilution) to stimulate the [^{3}H]thymidine incorporation of IL-2-dependent CTLL-2 cells (^{3}H cpm) was measured. Each measurement was made in triplicate.

L929 cells were infected or mock infected for 22 h prior to papain treatment to permit the analysis of class I MHC expression during a period when MAV-1 early proteins are abundant and any inhibitory effects due to infection would be likely to be operating. Twenty-two hours postinfection is near the end of the early phase of MAV-1 infection (2). E1A and E3 mRNAs are first detectable at 14 h postinfection (2, 4). The E3 gp11K protein is detected as early as 16 h postinfection, peaking between 24 and 28 h postinfection (5). Papain treatment affected mock- and MAV-1-infected cells equivalently, resulting in 83 and 85% reductions in surface antigen compared with the untreated cells, respectively (Fig. 4A). Following papain treatment, cells were returned to growth media and cultured for 0.5, 1, 1.5, or 2 h to permit regeneration of surface antigen. The levels of regenerated surface antigen on mock-infected and infected cells were equivalent at every recovery period tested, and the data from one experiment are shown (Fig. 4B). At 0.5 h of recovery, the amount of antigen was similar to that at 1 h recovery (data not shown). The surface antigen levels after 1, 1.5, or 2 h of recovery were 21, 41, and 52% of untreated controls for mock-infected cells and 18, 35, and 57% of untreated controls for infected cells, respectively.

Class I MHC-associated antigen presentation to T cells is not altered by MAV-1 infection. To provide a functional assay of the effect of MAV-1 infection on the class I MHC presentation of antigen to T cells, we analyzed the ability of a CTL hybridoma to recognize MHC-associated peptide on the surfaces of infected cells. Mock- and MAV-1-infected 5AKα3, C57SV, and SVB6KH cells (all *H-2K*^{b}) were incubated with ovalbumin peptide and used to stimulate IL-2 production by H-2K^b-ovalbumin-specific T-cell hybridomas. The growth of the CTLL-2 cells, quantitated by [^{3}H]thymidine incorporation, is a measure of the IL-2 activity present in the T-cell supernatant and provides a functional assay of the H-2K^b expression of and peptide presentation by 5AKα3, C57SV, and SVB6KH. Mock- and MAV-1-infected 5AKα3, C57SV, and SVB6KH cells stimulated IL-2 production from the ovalbumin-specific hybridoma cells to the same degree (Fig. 5 and data not shown).

DISCUSSION

Representatives of human adenovirus subgenera A through E decrease class I MHC cell surface antigen expression levels (14, 27, 37). It has been proposed that this decrease allows adenovirus-infected cells to escape CTL-mediated immune surveillance, thus permitting viral persistence (48). Because of the host-specific nature of adenoviruses, it has been difficult to assess how important a reduction in levels of class I MHC cell surface antigen is to viral persistence. We expected that MAV-1, like human adenovirus, would reduce the surface expression of the class I MHC antigens, allowing us to analyze the role of this interaction in MAV-1 pathogenesis and persistence. Surprisingly, rigorous testing of the surface antigen expression levels of 10 different class I MHC alleles showed no difference between mock- and MAV-1-infected cells (Fig. 1 and 2 and Table 1). The Ad2/5 E3 gp19K protein has different binding affinities for class I MHC allotypes, and cells of distinct class I MHC haplotypes vary in the extent to which the E3 gp19K protein reduces class I MHC surface expression (9, 39, 43). It seems likely that if MAV-1 infection induces the downregulation of class I MHC surface expression, at least one of the class I MHC types tested would have been susceptible to this effect. Routes and Cook have proposed that the cell type or duration of infection is more relevant to the ability of the E3 gp19K protein to decrease class I MHC surface antigen levels than is the MHC haplotype (35). In this study, seven different cell lines (representing cells of renal, renal adenosarcoma, connective tissue, and embryonic origin) were examined. The time points of infection extended well into the late stages of infection, when cell death was imminent. Our results imply that cell types in which MAV-1 infection decreases class I MHC surface expression are rare or nonexistent.

It is possible that MAV-1 infection resulted in only a slight change in the overall levels of class I MHC surface expression, which would be undetectable in a background of stable antigens on the cell surface. However, the experiments in which cells were treated with BFA demonstrated that inhibition of cellular membrane transport and thus protein processing (analogous to the block in class I MHC glycosylation effected by the Ad2/5 E3 gp19K protein) brought about a clearly detectable decrease in class I MHC antigen levels on the cell surfaces even following a relatively short period (2 h) of treatment. These results argue against the possibility that the class I antigens present on the surface prior to infection remained at high levels and thus obscured detection of the effects of infection on the synthesis, transport, and surface expression of newly synthesized class I antigens.

Mock- and MAV-1-infected cells regenerated newly synthesized class I antigens on the cell surface with equal kinetics following proteolytic removal with papain, thus demonstrating that infection did not block the expression of class I antigens. There was no significant difference between cell viabilities of the mock- and MAV-1-infected cell populations treated with papain (data not shown). Thus, the regeneration of surface class I antigens seen in the infected cells was not due to preferential recovery of a subpopulation of uninfected cells in the culture.

We also investigated the possibility that while there was no apparent decrease in the amount of class I MHC surface antigen on infected cells, antigen presentation by class I MHC might still be functionally impaired. MAV-1-infected and uninfected cells

stimulated class I MHC-restricted T-cell hybridomas equally, indicating that infection did not effect a functional difference in the class I MHC antigen-mediated presentation. This result is further supported by studies in which immune spleen cells from mice immunized with MAV-1 elicited the specific cytolysis of MAV-1-infected mouse embryonic or lymphoid cells (21).

The ability of MAV-1 to persist cannot be explained by a general mechanism in which adenoviral infection inhibits the class I MHC presentation of antigen to CTL. The results presented here suggest two possible models for MAV-1-induced viral persistence. One model is that a unique cell type exists in which MAV-1 infection down regulates class I MHC surface expression. For human adenoviral infections, the E3 gp19K-mediated decrease in class I MHC surface expression may not be a general mechanism but may operate only in a small population of (293-like) cells within an individual, forming a focus of viral persistence (35). While endogenous expression of MAV-1 E1A activity did not induce a decrease of class I MHC antigen levels in MAV-1-infected cells in our studies, it remains possible that we did not analyze the unique cell type in which this mechanism could operate.

An alternative model is that decreased class I MHC surface expression is not important to the ability of MAV-1 to persist and that another mechanism is active. Support for this model comes from studies with recombinant vaccinia viruses that express the Ad2 E3 gp19K protein. Mice infected with these recombinant viruses exhibit no differences in viral replication or lethality and no alteration of CTL or natural killer cell responses compared with mice infected with recombinant vaccinia viruses containing either inverted or mutated copies of the E3 gp19K coding sequence (11, 17). These results indicate that the demonstrated ability of the E3 gp19K protein to decrease the surface expression of class I MHC antigens in vitro may not be indicative of an ability to significantly alter the cell-mediated immune response to an infection in vivo. More insight might be gained when the types of cells that support MAV-1 persistence are identified and their class I MHC expression characteristics are analyzed. Studies that define the site of MAV-1 persistence in vivo are necessary for the resolution of these questions.

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