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Human Parainfluenza Virus Type 3 Up-Regulates Major Histocompatibility Complex Class I and II Expression on Respiratory Epithelial Cells: Involvement of a STAT1- and CIITA-Independent Pathway

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Human parainfluenza virus type 3 (HPIV3) infection causes severe damage to the lung epithelium, leading to bronchiolitis, pneumonia, and croup in newborns and infants. Cellular immunity that plays a vital role in normal antiviral action appears to be involved, possibly because of inappropriate activation, in the infection-related damage to the lung epithelium. In this study, we investigated the expression of major histocompatibility complex (MHC) class I and II molecules on human lung epithelial (A549) and epithelium-like (HT1080) cells following HPIV3 infection. MHC class I was induced by HPIV3 in these cells at levels similar to those observed with natural inducers such as beta and gamma interferon (IFN-β and -γ). MHC class II was also efficiently induced by HPIV3 in these cells. UV-irradiated culture supernatants from infected cells were able to induce MHC class I but not MHC class II, suggesting involvement of released factors for the induction of MHC class I. Quantitation of IFN types I and II in the culture supernatant showed the presence of IFN-β as the major cytokine, while IFN-γ was undetectable. Anti-IFN-β, however, blocked the HPIV3-mediated induction of MHC class I only partially, indicating that viral antigens, besides IFN-β, are directly involved in the induction process. The induction of MHC class I and class II directed by the viral antigens was confirmed by using cells lacking STAT1, an essential intermediate of the IFN signaling pathways. HPIV3 induced both MHC class I and class II molecules in STAT1-null cells. Furthermore, MHC class II was also induced by HPIV3 in cells defective in class II transactivator, an important intermediate of the IFN-γ-mediated MHC class II induction pathway. Together, these data indicate that the HPIV3 gene product(s) is directly involved in the induction of MHC class I and II molecules. The induction of MHC class I and II expression by HPIV3 suggests that it plays a role in the infection-related immunity and pathogenesis.

Human parainfluenza virus type 3 (HPIV3), a paramyxovirus, is an important respiratory pathogen and a major cause of bronchiolitis and pneumonia in newborns and infants (12, 19, 31, 38, 42). Reinfection and persistent infection with the virus have been documented in various clinical situations (1, 15, 32, 33). These characteristics are different from those of other paramyxoviruses such as measles and mumps viruses, because infection with those viruses results in the development of lifelong immunity (37). The HPIV3 infection is often associated with severe damage to the lung epithelium. A complex interaction between the virus and the host immune system, the cell-mediated immune response, and the virus-specific immunoglobulin E antibody response most likely play a role in the lung epithelial cell damage (10). The cell-mediated immune response by up-regulating the expression of major histocompatibility complex (MHC) molecules has previously been reported for paramyxoviruses, namely, respiratory syncytial virus and measles virus (11, 30). MHC class I and II molecules are cell surface glycoproteins that are involved in the antigen presentation arm of the immune response. MHC class I molecules are involved in the processing of endogenous antigens and presenting the antigen-derived peptides to $CD8^+$ T cells, usually cytotoxic T lymphocytes (CTL). They are ubiquitously expressed, and their basal level of expression can be induced by a number of cytokines and viral factors (16). In particular, beta and gamma interferon (IFN-β and -γ) are the potent inducers of MHC class I molecules, and STAT1 (signal transducer and activator of transcription) plays an essential role in the activation of MHC class I transcription (14). MHC class II molecules, on the other hand, are involved in the processing of exogenous antigens and presenting the processed antigens to the $CD4^+$ T cells, usually T helper cells. This results in the activation of T cells which are involved in various functions, including (i) the direct lysis of virus-infected cells through the activity of CTL and (ii) production of cytokines that may either activate other cells of the immune system (macrophage, B cells, and CTL) or interfere with virus replication (11, 16, 30). Unlike the expression of MHC class I molecules, the constitutive expression of MHC class II molecules is restricted primarily to B cells, dendritic cells, thymic epithelium, and macrophages. The appropriate, constitutive, and inducible expression of MHC class II is essential for normal immune function, whereas aberrant expression in various tissues has been implicated in the pathogenesis of autoimmune diseases such as rheumatoid arthritis, autoimmune nephritis, insulin-dependent diabetes mellitus, inflammatory bowel disease, and multiple sclerosis (25, 27). Specific epithelium-like cells in various tissues can be induced to express MHC class II molecules upon exposure to IFN-γ, a potent inducer. The recently identified class II transactivator (CIITA) has been shown to be essential for activation of transcription of MHC class II genes in this process (34). Besides IFN-γ, other cytokines such as tumor necrosis factor alpha and interleukin 4 and other agents such as viral antigens

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have been shown to induce MHC class II (2, 16, 22, 28, 41). The expression of class II molecules by human bronchial epithelial cells following IFN-γ induction or virus infection suggests that these cells may be involved in mucosal immunity as well as infection-related immunopathology (16, 40). In this context, how class II antigen expression on epithelial cells is modulated by cytokines or directly by the viral antigens remains unclear. Also, whether epithelial cells bearing class II molecules can function as antigen-presenting cells (APC) remains unknown.

In this work we have investigated the effect of HPIV3 on the antigen-presenting macromolecules', i.e., the MHC class I and II molecules', expression on infected cell surface. Our data clearly demonstrate that cell surface expression of both MHC class I and II molecules are up-regulated in A549 as well as HT1080 cells following HPIV3 infection. The induction of MHC class I was mediated through the production of IFN-β and also by the direct interaction of viral antigens. The MHC class II induction, on the other hand, was directly mediated by the viral antigens. The induction of MHC class I and II molecules also occurred on cells lacking the IFN signaling intermediate STAT1, indicating a direct effect of viral antigens in the induction process. These results suggest that cell-mediated immune response involving MHC molecules plays an important role in HPIV3 infection-related immunity and pathogenesis.

MATERIALS AND METHODS

Biological reagents. Human recombinant IFN-β was purchased from Biosource International (Camarillo, Calif.). Human recombinant IFN-γ was purchased from Boehringer Mannheim (Indianapolis, Ind.); poly(I)-poly(C) was purchased from Pharmacia Biotech (Piscataway, N.J.).

Cell lines and culture conditions. CV-1 (African green monkey kidney) cells were used for growing the virus and for plaque assays. A549 is a lung adenocarcinoma cell line (ATCC CCL 185) which has been used as a model of type II alveolar epithelial cells. HT1080 (ATCC CCL 121) is a fibrosarcoma cell line. The 2fTGH cell line was derived from the HT1080 cell line. U3A is a STAT1 mutant cell line, and G3A is a CIITA-defective cell line; both of them are derived from 2fTGH (4, 6, 29). 2fTGH, U3A, and G3A cell lines were gifts from George Stark (Department of Molecular Biology, Lerner Research Institute, Cleveland Clinic Foundation). All the cell lines mentioned above were maintained in Dulbecco's modified Eagle medium containing 1% L-glutamine, 1% penicillin-streptomycin, and 10% fetal bovine serum.

Virus stock and infection. The HPIV3 viral stock HA-1, National Institutes of Health catalog no. 47784, was grown in the CV-1 cell line. For the MHC class I assay, the virions released in the culture medium were purified by centrifugation at 10,000 × *g* to remove cell debris followed by ultracentrifugation at 100,000 × *g* for 2 h at 4°C with a SW 50.1 rotor, as described previously (7). The purified virus pellet was suspended in buffer containing 50 mM HEPES-KOH (pH 7.5) and 50 mM NaCl, and the purity was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie blue staining. No IFNs were detected in the purified virus pool by antivirus bioassay and enzyme-linked immunosorbent assay (ELISA). For the MHC class II assay, infected CV-1 cell supernatants were used as virus stocks. Titers of both viral sources were determined by plaque assay. The unpurified stock contained virus at 2×10^6 PFU/ml, and the purified stock contained virus at 10^8 PFU/ml. For some experiments, the virus particles were inactivated with UV light as previously reported (11).

Experiments were performed by infecting the cells (5×10^5) with HPIV3 at a multiplicity of infection (MOI) of 1 in a total volume of 2 ml in a 12-well plate at 37°C, and the cells were harvested at 48 and 72 h postinfection for MHC class I and II assays, respectively. The virus infection was conducted in the same medium used for growing the cells. For the supernatant transfer experiments, the culture supernatants were collected at 48 h postinfection and transferred (20% volume) to fresh monolayers of corresponding cell types. The cells were harvested after 48 and 72 h for MHC class I and II assays, respectively.

Transient transfection. Both A549 and HT1080 cells were transfected with poly(I)-poly(C) by a FuGENE 6 transfection technique (Boehringer Mannheim) according to the manufacturer's protocol. Various doses of poly(I)-poly(C) ranging from 50 to 150 μg/ml and control medium were added to cells in the absence or presence of FuGENE 6 reagent in 10% fetal bovine serum containing Dulbecco's modified Eagle medium. Cells were cultured for 48 and 72 h and assayed by flow cytometry for MHC class I and II molecules, respectively.

Flow cytometry. The cells were plated at 5×10^5 cells/well into 12-well plates, and after 12 h the cells were infected with HPIV3 (MOI, 1.0); mock-infected cells served as the control. After indicated times postinfection, the cells were removed by short treatment with 0.1 M EDTA, washed twice in phosphate-buffered saline (PBS), and counted. Viability was determined by trypan blue dye exclusion. The cells were incubated with specific antibodies or the same isotype used for the control (1 to 2 μg/sample, according to the manufacturer's protocol) in a 100-μl reaction mixture containing 1× PBS, 1% bovine serum albumin, and 0.01% sodium azide for 30 min at room temperature. Flow cytometry analysis of the cells was performed on a FACScan device (Becton-Dickinson; San Jose, Calif.) with Cyclops software (Cytomation, Fort Collins, Colo.). Ten thousand cells were analyzed for each sample. In addition to an ungated analysis, a gate was set (on the basis of the dot plot for 90° light scatter versus forward-angle light scatter) to exclude any cell debris or dead cells from the analysis. The antibody used in the staining for human MHC class I is murine monoclonal antibody (MAb) to HLA-ABC (W6/32) conjugated directly to fluorescin isothiocyanate (FITC) obtained from Biodesign, New York, N.Y. The antibody used in the staining for human MHC class II is murine MAb to HLA-DR, L243, conjugated directly to FITC (Becton Dickinson). For the dual color assay for MHC class I and HPIV3, the antibody used in the staining for human MHC class I antigen is murine MAb to HLA-ABC (W6/32) conjugated directly to phycoerythrin (Biodesign). The antibody used in the staining for human MHC class II antigen is murine MAb to HLA-DR, L243, conjugated directly to phycoerythrin (Becton Dickinson). The antibody used for HPIV-3 is a MAb specific for HPIV3 surface glycoproteins conjugated directly to FITC (Biodesign). Nonspecific background staining was determined with a control FITC-conjugated isotype-matched Ab (Becton Dickinson).

Cytokine assays. IFN-γ was assayed by ELISA (R&D systems, Minneapolis, Minn.), and IFN-β was assayed by ELISA (Biosource International). The presence of IFN-α/β in the culture supernatant of HPIV3-infected cells was determined by analyzing their ability to inhibit vesicular stomatitis virus-induced cytopathic effect on WISH cells, as described previously (21). Briefly, A549 and HT1080 cells were infected with HPIV3 at an MOI of 1.0 at 37°C and the culture supernatant was collected at 48 h postinfection. Culture supernatant of uninfected cells served as the control. The culture medium was first exposed to UV light to inactivate the released virions and then used to measure activity against vesicular stomatitis virus. Sheep anti-IFN-α polyclonal Ab, sheep anti-IFN-β polyclonal Ab (Biosource International), and sheep serum control were used to quantitate both IFN-β and IFN-α in the cell supernatants. The assay was standardized with reference to IFN of known activity. Cell viability was measured by staining wells with neutral red in PBS and elution in 50% ethanol in 0.1 M NaH_2PO_4, and the absorbance at 540 nm was determined. The results are presented as percent protection, calculated as (A_{540} of the sample − A_{540} of the virus control)/(A_{540} of the cell control) − A_{540} of the virus control) × 100.

RESULTS

Induction of MHC class I and II expression on A549 and HT1080 cells by HPIV3. Infection of cells by several paramyxoviruses has been shown to up-regulate the expression of MHC class I and II molecules, which are important components of the host immune response to these viruses (11, 26, 30). Up-regulation of the expression of MHC molecules by these viruses has been suggested to be involved in the infection-related immunopathology. To understand the molecular mechanism of HPIV3 infection-related immunopathology, manifested by the damage of respiratory tract epithelium, we examined the effect of HPIV3 on the expression of MHC class I and II molecules on human respiratory epithelial cells, A549. These are human lung adenocarcinoma cells that have been used as a model of type II alveolar epithelial cells and are susceptible to infection with HPIV3 (43). We also used another human epithelium-like cell line, HT1080, derived from fibrosarcoma and originally subcultivated under conditions that eliminate the growth of fibroblasts and favor that of epithelial cells (17). HT1080 cells were also found to be susceptible to infection with RNA viruses (17). Furthermore, several mutant cell lines have been generated from the parental HT1080 cells, and those have been extensively used for characterizing the components of the IFN signaling pathway, e.g., JAK (Jenus kinase) and STAT (6). The use of HT1080 cells would therefore not only help determine the effect of HPIV3 on MHC class I and II expression, but also enable us to delineate the molecular mechanism of regulation of expression of MHC molecules by using various mutant cell lines. The A549 and HT1080 cells were infected with HPIV3 at an MOI of 1, and the cells were harvested at 48 h postinfection for MHC class I and 72 h

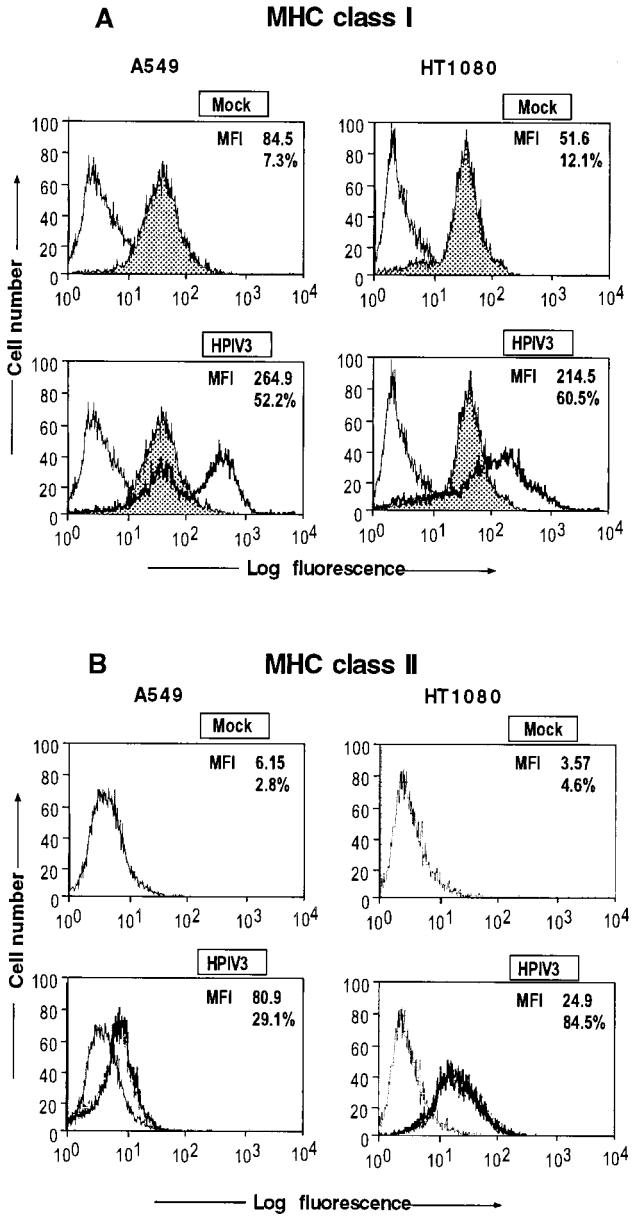


FIG. 1. Flow cytometric analysis of MHC class I (A) and class II (B) expression on A549 and HT1080 cells following HPIV3 infection. The A549 and HT1080 cells (5×10^5 cells) were infected with HPIV3 (MOI, 1.0) and were harvested at 48 h postinfection for MHC class I assays and at 72 h postinfection for MHC class II assays. Mock-treated uninfected cells (Mock) served as the control. In each panel, the MFI and the percentage of cells staining for MHC class I or class II are indicated. Results are representative of four independent assays.

postinfection for MHC class II assays. The cells were viable by more than 90%, as determined by trypan blue staining. These cells were processed for flow cytometry by using HLA-ABC and HLA-DR antibodies, and the fold increase of mean fluorescence intensity (MFI) was determined. As shown in Fig. 1, cell surface expression of MHC class I was increased, following HPIV3 infection, by about 3-fold on A549 cells and 4-fold on HT1080 cells, while class II expression was increased by about 13-fold on A549 cells and 7-fold on HT1080 cells. These results clearly indicate that HPIV3 upregulates the cell surface expression of both MHC class I and II on epithelial cells.

Since, IFNs are the natural inducers of MHC class I and II molecules during antiviral response, we examined the induction of MHC molecules on these cells following IFN-γ treatment and compared this to the induction observed with HPIV3. Similarly, induction by IFN-β was examined because its involvement in the induction of MHC class I expression has been previously demonstrated (11). As shown in Fig. 2, MHC class I expression on A549 and HT1080 cells was induced by about eightfold on A549 and fourfold on HT1080 cells following treatment with 100 U of IFN-β/ml. Similarly, 100 U of IFN-γ/ml induced MHC class I expression on A549 by about sevenfold and induced that on HT1080 by about sixfold. MHC class II expression, however, was not induced on A549 cells following IFN-γ treatment, as reported previously by other laboratories (36), whereas MHC class II expression on HT1080 was induced by about 16-fold after 72 h. The level of HPIV3-mediated induction of MHC class I and II expression is therefore comparable to the level of induction by the natural inducers, IFN-β and IFN-γ.

Production of infectious virions by A549 and HT1080 cells and their role in MHC class I and II expression. To determine whether both A549 and HT1080 cells supported the replication of HPIV3, we examined the production of infectious virions from these cells. The cells were infected with HPIV3 at an MOI of 1.0, and at various times (24, 48, and 72 h) postinfection the number of progeny virions released into the medium was determined by plaque assay. As shown in Table 1, both A549 and HT1080 cells supported efficient replication of HPIV3.

The roles of viral gene products in the induction of MHC class I and II were examined by double immunofluorescent labeling and flow cytometry. As shown in Fig. 3, MHC class I expression occurred on both the cell population containing the viral antigens and that without the viral antigens, suggesting a role of soluble extracellular factors and possibly also viral gene products. MHC class II expression, on the other hand, occurred on the cell population containing the viral antigens, suggesting that the viral gene products are the major regulator of the induction of MHC class II in infected cells.

To determine whether infectious virions were required for the induction of MHC class I and II, we inactivated the virions by exposure to UV light before infection. The absence of infectious replicating virions in the UV-treated supernatant was confirmed by plaque assay. Levels of expression of MHC class I on A549 and HT1080 cells were determined at 48 h postinfection, whereas MHC class II expression was determined at 72 h postinfection. MHC class I expression was induced by about 13-fold on A549 cells and 14-fold on HT1080 cells by UV-inactivated virus particles (data not shown). Furthermore, the induction level was comparable to that observed with 100 U of IFN-β (Fig. 2). These results suggest that the HPIV3-mediated induction of MHC class I possibly resulted by the interaction of viral envelope glycoproteins which can induce IFN production, as reported previously for other viruses (8). MHC class II induction, on the other hand, was abrogated by UV inactivation of the virus particles, indicating a requirement of infectious particles in this induction process (data not shown).

Since nonsegmented negative-strand RNA viruses are known to generate double-stranded RNA during replication, which in turn induces IFN, we examined the effect of various doses (50 to 150 μg) of poly(I)-poly(C), which is used to mimic intracellular double-stranded RNA. MHC class I expression occurred on both A549 and HT1080 cells following poly(I)-poly(C) treatment, indicating that double-stranded RNAs are possibly involved in MHC class I induction (data not shown).

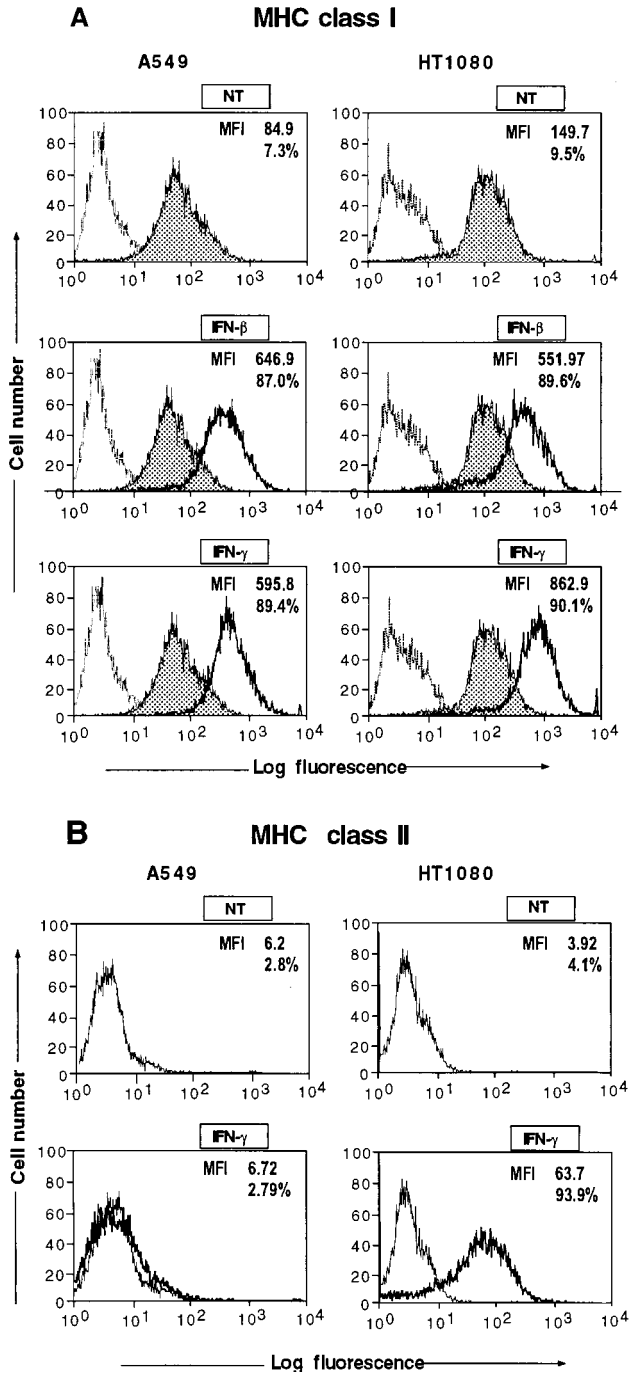


FIG. 2. Flow cytometric analysis of MHC class I (A) and class II (B) expression on A549 and HT1080 cells induced by IFN-β and IFN-γ. The A549 and HT1080 cells (5×10^5 cells) were induced with or without IFN-β (100 U/ml) and IFN-γ (100 U/ml) and were harvested after 48 h of treatment for MHC class I and after 72 h treatment for MHC class II assays. In each panel, the MFI and the percentage of cells staining for MHC class I or II are indicated. Results are representative of four independent assays. NT, no treatment.

By contrast, MHC class II induction was not detected in either of the cell lines following poly(I)-poly(C) treatment (data not shown). These data suggest that the induction of IFN by the viral replication intermediate, double-stranded RNA, is involved, at least in part, in the induction of MHC class I. The pattern of MHC class II induction, however, suggests that some viral protein(s) directly induces the MHC class II, as reported for measles virus (30). Alternatively, viral protein(s) may induce some other cytokines that in turn induce MHC class II (11).

TABLE 1. Production of infectious HPIV3 virions from A549 and HT1080 cells

Time (h)	Virus yield (PFU/ml)[a]	
	A549	HT1080
24	1×10^5	1×10^5
48	1×10^6–1×10^7	1×10^6–1×10^7
72	5×10^6–1×10^7	5×10^6–1×10^7

[a] A549 and HT1080 cells were infected with HPIV3 at an MOI of 1.0, and at the indicated times postinfection the titers of the progeny virions released into the medium were determined by plaque assay on a monolayer of CV-1 cells.

MHC class I, but not MHC class II, can be induced with the culture supernatant from infected cells. To investigate the involvement of soluble factors such as IFN-β and IFN-γ in the induction of MHC molecules, specifically MHC class I, we transferred the culture supernatant from infected A549 and HT1080 cells to fresh monolayers of corresponding cell types. The A549 and HT1080 cells were harvested at 48 and 72 h postinfection, respectively, and MHC class I and II expression was determined. As shown in Fig. 4, supernatant from infected A549 cells induced the MFI for MHC class I expression by 3.5-fold on fresh A549 cells, while supernatant from infected HT1080 cells induced this expression by 3-fold on fresh HT1080 cells. MHC class II expression, on the other hand, was not induced on fresh monolayers of A549 and HT1080 cell types by the culture supernatants from corresponding infected cells (data not shown). These data indicate that MHC class I expression can be transferred with the culture supernatant, perhaps due to the presence of IFNs, whereas MHC class II expression cannot be transferred.

Although induction of IFN by some paramyxoviruses in pulmonary epithelial cells has been reported (11), such induction of IFN synthesis by HPIV3 has not been previously investigated. Therefore, we examined the presence of IFN types I and II in the culture supernatant from infected A549 and HT1080 cells. We first tested the supernatant for the presence of immunoreactive IFN-γ by a sensitive ELISA and observed, as expected, that IFN-γ was not detected in the culture supernatant of both A549 and HT1080 cells, in neither control nor infected cultures (data not shown). When IFN-β production was similarly determined, the culture supernatant from infected A549 cells was found to contain about 500 U of IFN-β/ml, while that from HT1080 cells contained about 300 U of IFN-β/ml at 48 h postinfection. By antivirus bioactivity assay, IFN-β was found to be 80% and IFN-α was 20% of the total IFN type I in the culture supernatant. Consistent with these data, as shown in Fig. 4, neutralizing anti-IFN-β inhibited a greater part of the culture supernatant-mediated induction of MHC class I in both cell types, whereas anti-IFN-α had no effect (data not shown), indicating the involvement of IFN-β in the culture supernatant-mediated induction of MHC class I. Next, we examined whether the HPIV3-mediated induction of MHC class I could also be inhibited by anti-IFN-β, if added to the culture medium during infection. The anti-IFN-β inhibited the HPIV3-mediated induction of MHC class I only partially (~50%) (data not shown). Together, these results indicate that the induction of MHC class I in HPIV3-infected cells is mediated partly by IFN-β and partly by the viral antigens, either directly or through the production of some other cytokines.

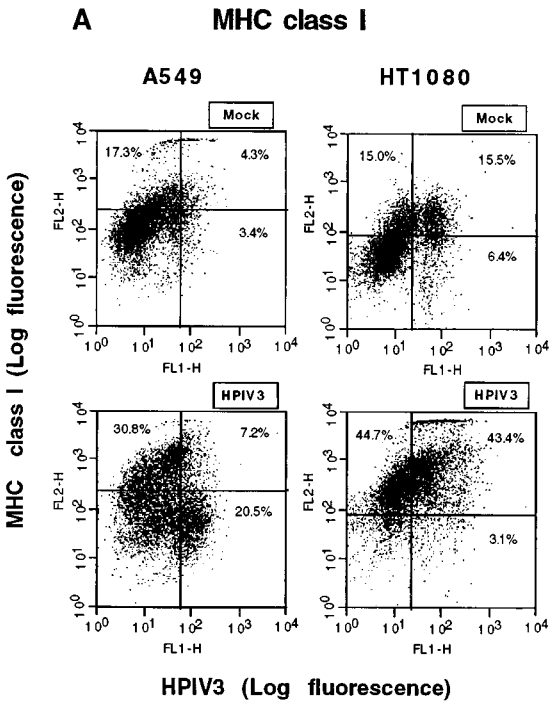
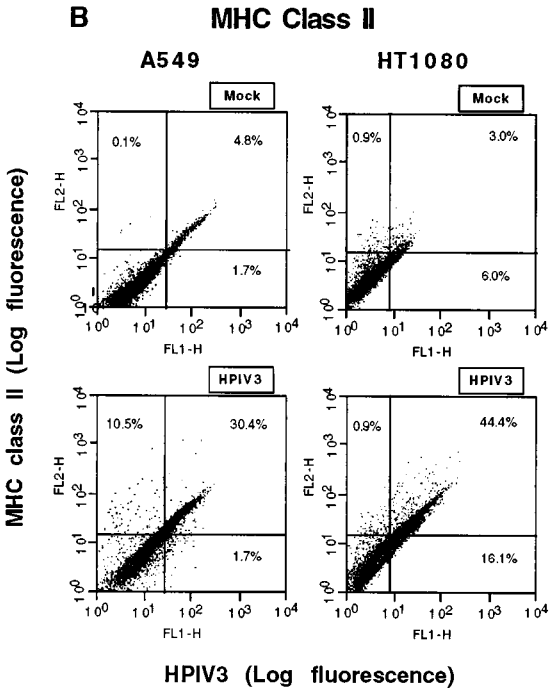


FIG. 3. Dual color staining analysis for MHC class I and HPIV3 (A) and for class II and HPIV3 (B) antigen expression on A549 and HT1080 cells. A549 and HT1080 cells (5×10^5) were infected with HPIV3 (MOI, 1.0), and the cells were harvested at 48 h postinfection for dual color staining for MHC class I and HPIV3 and at 72 h postinfection for dual color staining for MHC class II and HPIV3. Results are representative of three independent assays. Mock, mock-treated, uninfected cells.



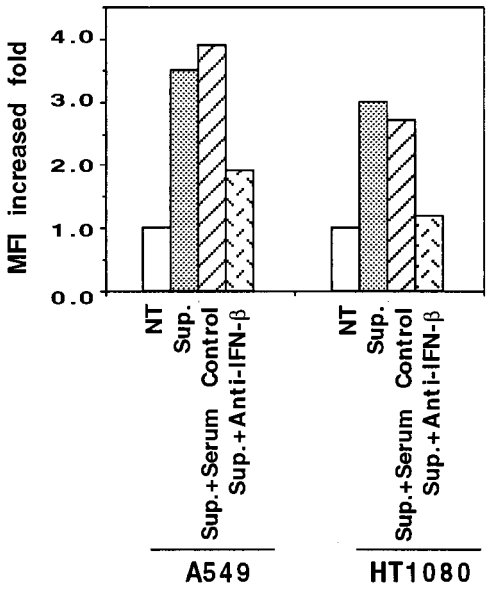


FIG. 4. Flow cytometric analysis for MHC class I expression on A549 and HT1080 cells transferred with supernatants from corresponding HPIV3-infected cell types. The culture supernatants (Sup.) from HPIV3-infected cells, A549 and HT1080, harvested at 48 postinfection were inactivated by UV and transferred to fresh monolayers of corresponding cell types with or without anti-human IFN-β Ab (1,000 U/ml) or sheep serum control. The A549 and HT1080 cells were harvested at 48 and 72 h postinfection, respectively, to determine the increase in MHC class I and II expression. Results are expressed as fold MFI increase and are representative of three independent assays. NT, no treatment.

HPIV3 induces MHC class I and II expression on STAT1-null and CIITA-defective cells. To gain insight into the molecular mechanism of the induction of MHC class I and class II by HPIV3, we used STAT1-null cells. STAT1 has been shown to be an essential component of both IFN type I and type II signal transduction pathways, being involved in the activation of transcription of IFN-responsive genes following its phosphorylation by JAK in response to IFNs (6, 35). The STAT1-null cell line (U3A) is therefore defective in both IFN-α/β and IFN-γ signal transduction and thus provided us a model for the primary cell response to virus, without endogenous IFN autocrine effect.

First, the U3A and the parental cells 2fTGH (derived from HT1080) were treated with IFN-β and IFN-γ to confirm the defect in the signal transduction pathway. As shown in Fig. 5A, IFN-β and IFN-γ treatment of cells efficiently induced MHC class I on 2fTGH cells but failed to induce MHC on class I U3A cells. These cells were then infected with HPIV3 at an MOI of 1, and the cells were harvested at 48 h postinfection and analyzed for MHC class I expression. As shown in Fig. 5A, MHC class I was induced on 2fTGH and U3A cells, following HPIV3 infection, by about 6-fold and 3.5-fold, respectively. The culture supernatant from A549 cells which induced MHC class I on A549 cells by 3.5-fold (Fig. 4) was also tested in these cells and was found to induce MHC class I by 3-fold, as expected, only on the 2fTGH cells (Fig. 5A). These data indicate that in addition to the induction of MHC class I by IFN-β, the viral antigens also induce MHC class I through a pathway that does not require STAT1.

Next, the 2fTGH and U3A cells were treated with IFN-γ and induction of MHC class II was determined. As shown in Fig. 5B, MHC class II was induced by IFN-γ on 2fTGH cells by 2.5-fold, but no induction was observed on U3A cells. These results confirm the defect of U3A cells in signal transduction.

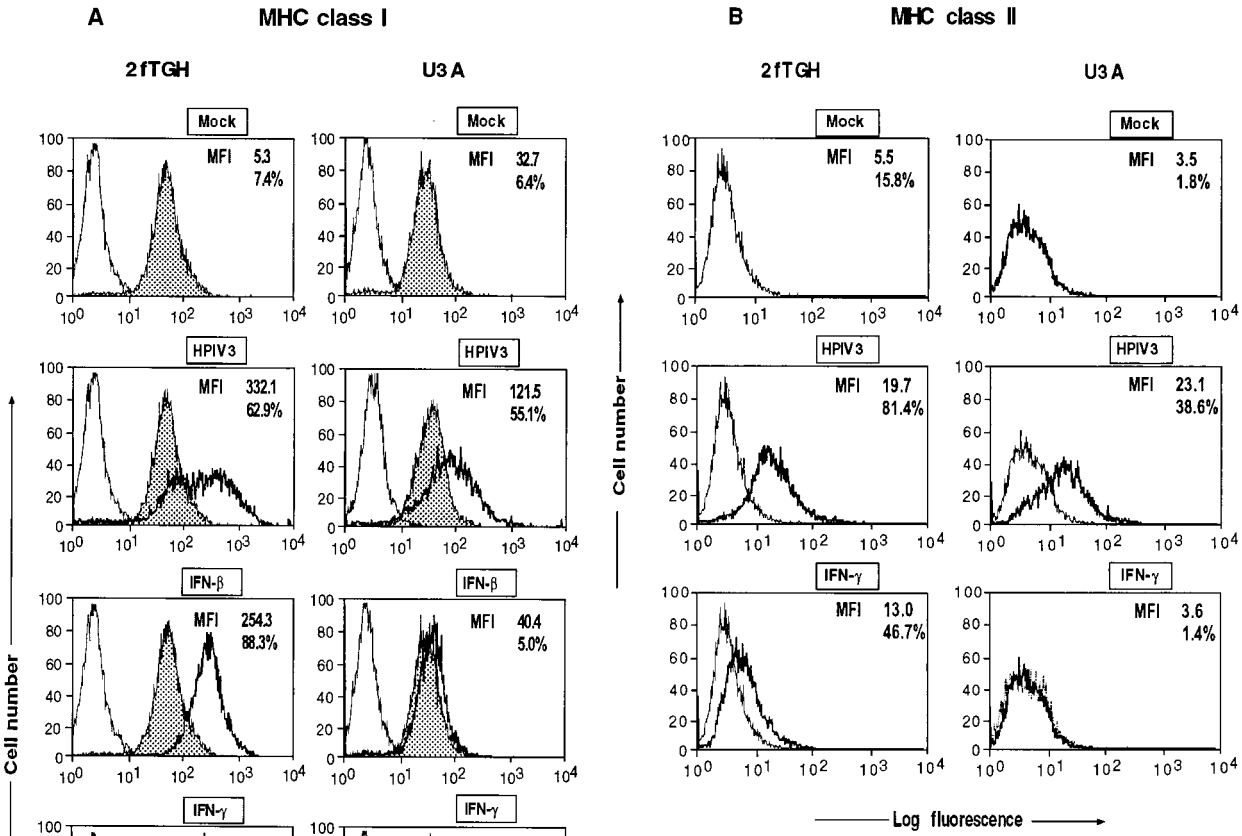


FIG. 5. MHC class I (A) and class II (B) expression on 2fTGH and U3A cells and comparison with that by IFN-β/γ. The mutant and parented cells (5×10^5) were induced with IFN-β (100 U/ml) (only for MHC class I expression) or IFN-γ (100 U/ml) or were infected with HPIV3 (MOI, 1.0). The cells were harvested after 48 and 72 h to determine the levels of MHC class I and II expression. In each panel, the MFI and the percentage of cells staining for MHC class I or II are indicated. Results are representative of four independent assays. Sup., supernatant.

When MHC class II induction by HPIV3 was examined, the parental cells, 2fTGH, as well as the mutant cells, U3A, expressed MHC class II at the same level (14-fold in 2fTGH cells and 11-fold in U3A cells). The level of induction was comparable to that observed with infected A549 and HT1080 cells. These data indicate that IFNs or other cytokine signaling pathways which require STAT1 are not involved in the activation of MHC class II by HPIV3. Thus, it appears that HPIV3 infection of human epithelial cells induces both MHC class I and II directly by viral proteins either interacting with the promoter or by activating some other pathway that hitherto remains uncharacterized.

Finally, to confirm the induction of MHC class II directly by viral antigens, we also investigated MHC class II induction on CIITA-defective cells (G3A). CIITA is the general regulator of MHC class II gene expression by IFN-γ (39). In the IFN-γ response, STAT1 is phosphorylated and translocates to the nucleus, where it binds to the GAS element of CIITA promoter IV and activates transcription of CIITA mRNA, which operates as the essential mediator of the MHC class II gene and its induction product (25, 39). Thus, the CIITA-defective cell line (G3A) is defective in the production of MHC class II by IFN-γ. We infected G3A as well as parental 2fTGH cells with HPIV3 at an MOI of 1 and examined MHC class II expression at 72 h postinfection. As shown in Fig. 6, MHC class II induction by HPIV3 occurred on both the parental (2fTGH) and CIITA-defective (G3A) cells at the similar levels. MHC class I induction by IFN-β, IFN-γ, and HPIV3 also occurred on these cells (data not shown), which is consistent with the previous findings that CIITA is not involved in the MHC class I induction. Together, these data indicate that HPIV3-mediated induction of MHC class I and II on pulmonary epithelial cells occurs through the direct interaction of viral antigens, which does not require the involvement of STAT1. Furthermore, MHC class I and II induction by HPIV3 can also occur in the absence of CIITA.

DISCUSSION

In this study we investigated the effect of HPIV3 infection of respiratory epithelial cells on MHC class I and II expression.

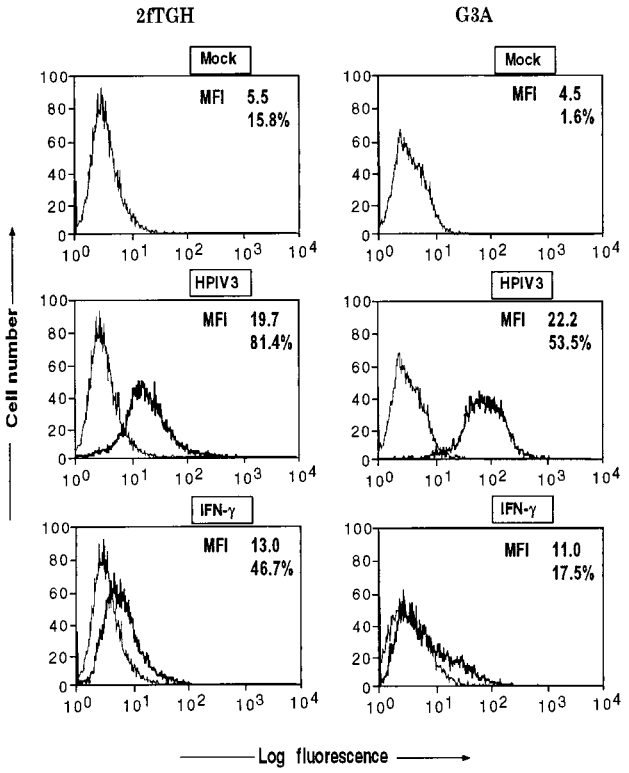


FIG. 6. MHC class II expression on 2fTGH and G3A cells by comparing with IFN-γ induction and with HPIV3 infection. These mutant cells (5×10^5) were induced with IFN-γ (100 U/ml) or infected with HPIV3 (MOI, 1.0). At 72 h postinfection, 2fTGH and G3A cells were harvested to determine the level of MHC class II expression. In each panel, the MFI and the percentage of cells staining for MHC class II are indicated. Results are representative of four independent assays. Mock, mock-treated, uninfected cells; Sup., supernatant.

Although the importance of MHC molecules in the immune response to HPIV3 in humans has not been adequately established, studies of Sendai virus infection in mice demonstrated the importance of both MHC class I and II in the recovery from pulmonary Sendai virus infection (23, 26). The present studies clearly demonstrate that HPIV3 induces MHC class I and II expression by the human respiratory epithelial cell line A549. This suggests that HPIV3-mediated induction of MHC class I and II accounts, at least in part, for the infection-associated immunopathology of the respiratory epithelium. MHC class I induction did not require infectious virions, whereas MHC class II induction occurred in a virus replication-dependent manner, indicating that viral proteins, perhaps envelope proteins in the case of MHC class I, are directly involved in this process. Double labeling of cells with anti-MHC class I and II and anti-HPIV3 followed by flow cytometric analysis confirmed that MHC class I expression was mediated by soluble factors as well as directly by viral antigens, whereas MHC class II induction occurred primarily by viral antigens. Furthermore, HPIV3 efficiently induced MHC class I and II on STAT1-null and CIITA-defective cells, indicating that the induction also occurs without the involvement of cytokine-mediated pathways that require STAT1 and CIITA, specifically in the case of MHC class II. These findings are therefore important, particularly in relation to HPIV3-mediated immunopathology in the airway epithelium.

Similar to those in our studies, several other viruses, including measles virus, respiratory syncytial virus, and cytomegalovirus, have been shown to up-regulate MHC class I or II molecules in infected cells (11, 18, 30). In some cases virus infection was found to increase the level of cytokine production, which in turn activated the MHC molecules. For example, in the case of respiratory syncytial virus infection, MHC class I was induced and the induction was found to be due to an increased synthesis of IFN-β (11). During human cytomegalovirus infection, on the other hand, the MHC class I molecules were directly stimulated by the viral antigens (18), whereas in the case of West Nile virus infection, both IFN-β and viral antigens were involved for the induction of MHC class I (9). In this respect, the HPIV3-mediated induction of MHC class I molecules resembles the West Nile virus-mediated MHC class I expression. The MHC class II molecules, on the other hand, appear to be directly mediated by the action of viral antigens.

The role of MHC molecules in processing and presentation of viral antigens to specific T cells for immune response has been well characterized. MHC class I-restricted T cells ($CD8^+$) function as CTL which, upon recognition of MHC-antigen complex, kill the target APC, e.g., virus-infected cells. MHC class II-restricted T cells ($CD4^+$), on the other hand, play several key roles in the generation of immune responses; they (i) stimulate antigen-activated class I-restricted CTL to kill virus-infected cells, (ii) stimulate antigen-activated B cells to produce antibodies, and (iii) activate cells of the nonspecific immune responses, e.g., macrophages, granulocytes, or eosinophils (13, 16). Some MHC class II-restricted T cells can also function as CTL and as such may have an important role in controlling certain virus infections, for example measles virus and herpes simplex virus infection (13). MHC class I molecules are ubiquitously expressed, and their basal level of expression can be induced by a number of cytokines and viral factors (16). MHC class II molecules are normally expressed on APC, but a variety of other cells, including epithelial cells, have been shown to produce MHC class II when activated by cytokines such as IFN-γ and other agents (2). In this respect, our data are the first demonstration that a nonsegmented negative-strand RNA virus induces MHC class II on epithelial cells in addition to MHC class I. However, it raises the question as to whether HPIV3-induced MHC molecules on epithelial cells participate in the immune response. Although priming of virgin CTL is believed to be mediated only by specialized APC, nonspecialized APC such as epithelial cells have also been shown to activate CTL (40). It has been estimated that an APC has to display a minimum of 2,000 antigenic complexes to be recognized by activated CTL (2, 13, 16, 41). The HPIV3-induced expression of MHC molecules seems to be competent for CTL activation, because the level of MHC class I and II expression is comparable to that observed with the natural inducers of these molecules, IFN-β and IFN-γ, respectively. Consistent with this, other viral infections that enhance the expression of MHC molecules have been shown to result in increased CTL lysis of infected cells (11). Further studies are needed to elucidate the mechanism of MHC class I and II induction by HPIV3 and its possible involvement in the activation of CTL.

The induction of MHC class I and II molecules on epithelial cells during HPIV3 infection may play an important role in the protective immune response of host against the virus by lysis of infected cells. Alternatively, this may lead to the development of infection-associated immunopathology by the lysis of both infected and neighboring mucosal epithelial cells that passively acquire released viral antigens. Previous findings, however, suggest a role of immunopathologic response in parainfluenza virus-mediated bronchiolitis (10). Cell-mediated immune response to parainfluenza viral antigens was found to be higher

among infants who developed bronchiolitis following HPIV3 infection than infants who developed only upper respiratory illness. Thus, induction of MHC class I and II on airway epithelial cells following HPIV3 infection possibly results in an increased CTL-mediated lysis of those cells and thus may contribute to the infection-associated immunopathology. Alternatively, the HPIV3-induced expression of MHC molecules may not be able to participate in the CTL-mediated lysis but rather may inhibit the lysis process. Thus, it may be a mechanism by which the virus evades the host immune surveillance. Further studies are, however, needed to gain a deeper understanding of the molecular mechanism of MHC class I and class II induction in HPIV3-infected airway epithelial cells and infection-associated immunopathologic response.

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