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This information is current as of September 16, 2021.

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J Immunol 2002; 169:1482-1491; ;
doi: 10.4049/jimmunol.169.3.1482
<http://www.jimmunol.org/content/169/3/1482>

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The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.



Effects of Rhinovirus Infection on Histamine and Cytokine Production by Cell Lines from Human Mast Cells and Basophils

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To understand the biochemical events that occur in the airways after rhinovirus (RV) infection, we developed for the first time a model in which the cell lines from human mast cells (HMC-1) and basophils (KU812) can be infected with RV14, a major group RV. Viral infection was confirmed by demonstrating that viral titers in culture supernatants, and RV RNA increased with time. RV14 infection alone and a combination of PMA plus calcium ionophore A23187, did not increase histamine production by these cells, although IgE plus anti-IgE increased the histamine production. However, histamine content in the supernatants increased in response to PMA plus A23187, or IgE plus anti-IgE after RV14 infection. PMA plus A23187 or IgE plus anti-IgE induced the production of IL-8 and GM-CSF in supernatants of HMC-1 cells and IL-4 and IL-6 in supernatants of KU812 cells. RV14 infection further increased the production of the cytokines, whereas RV14 infection alone did not alter the production of the cytokines by these cells. An Ab to ICAM-1 inhibited RV14 infection of the cells and decreased the production of cytokines and histamine after RV14 infection. RV14 infection enhanced the increases in intracellular calcium concentration and activation of NF- κ B by PMA plus A23187 in the cells. These findings suggest that RV14 infection may prime the cytokine and histamine production from mast cells and basophils and may cause airway inflammation in asthma. *The Journal of Immunology*, 2002, 169: 1482–1491.

Rhinoviruses (RVs)² are the major cause of the common cold and the most common acute infectious illness in humans (1). Furthermore, 80% of asthma exacerbations in school-aged children and half of all asthma exacerbations in adults are associated with viral upper respiratory infection, and the majority of viruses isolated are RVs (2, 3). Although several mechanisms have been proposed, it is still uncertain how viral respiratory infections cause an attack of wheezing in patients with asthma.

In contrast to a variety of other respiratory pathogens (e.g., influenza and adenovirus), cytotoxicity of epithelial cells does not appear to play a major role in the pathogenesis of RV infections (1). Instead, it is believed that the manifestations of RV-induced pathogenesis are the result of virus-induced mediators of inflammation (4). Infection of respiratory viruses including RVs activates histamine release from basophils of peripheral blood (5) and the plasma histamine content increases after RV infection (6). Furthermore, RV infection increases bronchial responsiveness to his-

tamine and ragweed Ag in association with increases in the histamine release from peripheral blood leukocytes in patients with allergic rhinitis (7). These findings suggest the important roles of histamine release in airway inflammation and bronchial hyperresponsiveness after RV infection. Mast cells are major sources of histamine release in airways and are associated with the pathogenesis of bronchial asthma (8). However, the effects of RV infection on histamine release from mast cells are still uncertain.

RV activates lymphocytes to induce IFN- γ production through a monocyte-dependent mechanism (9). Infection of epithelial cells with RV also induces production of several cytokines, such as IL-1 and TNF- α (4, 10, 11). Mast cells secrete proinflammatory factors including TNF- α (8, 12). These cytokines are known to mediate a wide variety of proinflammatory effects (13) and may play an important role in the pathogenesis of RV infections and bronchial asthma. Likewise, human mast cells and basophils produce cytokines in response to PMA and the calcium ionophore A23187 (14). However, the effects of RV infection on cytokine production in mast cells and basophils have not been studied.

In this study we investigated whether cell lines from human mast cells and basophils can be infected with RV14, a major group RV, and whether RV14 infection leads to increased production of histamine and proinflammatory cytokines.

Materials and Methods

Culture of HMC-1 and KU812 cell lines

HMC-1 cells, an immature human mast cell line, and KU812 cells, a human early basophilic leukocyte cell line, were cultured in T₂₅ tissue culture flasks (Corning Glass, Corning, NY) as previously described (15, 16).

Viral stocks

Stocks of RV14 were prepared from patients with common colds by infecting human embryonic fibroblast cells as previously described (11).

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Received for publication August 10, 2001. Accepted for publication May 17, 2002.

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² Abbreviations used in this paper: RV, rhinovirus; TCID, tissue culture-infective dose; [Ca²⁺]_i, intracellular calcium concentration.

Detection and titration of viruses

Detection and titration of RVs were performed by observing the cytopathic effects of viruses on human embryonic fibroblast cells with methods as previously described (11), and the amount of specimen required to infect 50% of the human embryonic fibroblast cells (tissue culture-infective dose (TCID₅₀)) was determined.

Viral infection of HMC-1 and KU812 cell lines

HMC-1 cells, suspended in 2 ml of IMDM supplemented with 10⁻⁵ M monothioglycerol, 10⁵ U/L penicillin, and 100 mg/L streptomycin, were infected with RV14 (10⁴ TCID₅₀ U/ml) for 1 h at 33°C. The cells were then rinsed in PBS and cultured in 2 ml of IMDM containing 2% ultra-low IgG FCS, 10⁻⁵ M monothioglycerol, and antibiotics at 33°C in a 5% CO₂ incubator.

To infect KU812 cells with RV14, medium was removed from confluent cells and was replaced with 2 ml of RPMI 1640. RV14 was added at a concentration of 10⁴ TCID₅₀ U/ml. After a 1-h incubation at 33°C, the viral solution was removed and the cells were rinsed with PBS. The KU812 cells were then cultured in RPMI 1640 containing 2% ultra-low IgG FCS and antibiotics at 33°C in a 5% CO₂ incubator.

To measure the time course of viral release, the whole volume of the medium was taken for the measurement of the viral content at 1, 24, and 48 h after RV14 infection and the same volume of fresh medium was replaced. Viral content in the supernatant is expressed as TCID₅₀ units per milliliter.

Detection of RV RNA and cytokine mRNA by RT-PCR

Detection of RV RNA and cytokine mRNA in HMC-1 and KU812 cells was performed with the RT-PCR method as previously described (11).

For each cytokine, mRNA expression in either HMC-1 cells or KU812 cells was examined before and at 24 h after RV14 infection. RV RNA production in either HMC-1 cells or KU812 cells was also examined before, and at 24 and 48 h after, RV14 infection.

Histamine assay

Histamine content of culture supernatants of HMC-1 and KU812 cells was measured as previously described (5, 17–19). The histamine release into the supernatant fluids containing cells and culture medium only was expressed as blanks. The percentage of intracellular histamine released was calculated as follows: % HR = (experimental – blank)/(complete – blank) × 100 (5, 18, 19).

Cytokine assays

We measured each cytokine of culture medium by specific ELISAs as previously described (11, 14, 20). We used an average value of replicate cultures from the same HMC-1 or KU812 cells (*n* = 3) for the analysis of cytokine production.

Effects of an Ab to ICAM-1 on RV14 infection and production of histamine and cytokines

To determine the effects of an Ab to ICAM-1 on RV14 infection and on the production of histamine and cytokines induced by RV14, cells were incubated for 30 min with medium alone or with medium containing either a mouse monoclonal anti-human Ab to ICAM-1 (84H10, 100 μg/ml; Immunotech, Marseille, France) or an isotype-matched mouse IgG1 control mAb (100 μg/ml; Chemicon International, Temecula, CA) at 37°C before RV14 infection as previously described (11).

Flow cytometry analysis of cell-membrane ICAM-1

ICAM-1 expression in either HMC-1 or KU812 cells was assayed by flow cytometry analysis as described previously (21). The cells were cultured for 24 h after RV14 or sham infection and then further stimulated for 24 h with PMA plus A23187.

Flow cytometry analyses of intracellular calcium concentration ([Ca²⁺]_i)

The [Ca²⁺]_i in HMC-1 and KU812 cells was measured with flow cytometry analysis as previously described (22). To examine the effects of RV14 infection on [Ca²⁺]_i, the cells were cultured for either 2, 6, or 24 h after RV14 infection. To examine the effects of RV14 infection on increases in [Ca²⁺]_i by PMA plus A23187, the cells were cultured for 24 h after RV14 infection. The cells were then further incubated in the medium containing both PMA and A23187 for 10, 30, or 60 min, 6 h, or 24 h.

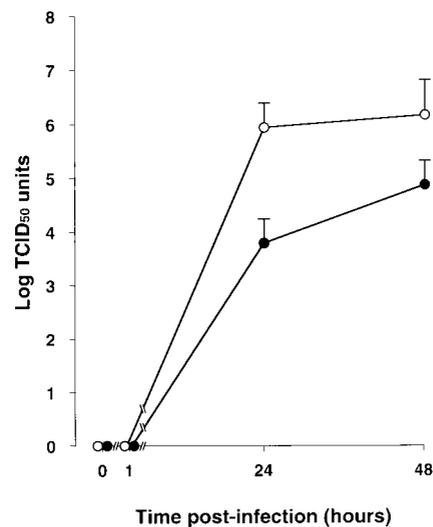


FIGURE 1. Viral titers in supernatants of either HMC-1 (○) or KU812 (●) cells during the first 1, 1–24, and 24–48 h after infection. Results are reported as means ± SEM from seven samples.

Isolation of nuclear extracts and EMSAs

Extraction of nuclei, EMSAs, and supershift assays were performed using methods described previously (4, 23).

Electron microscopic examination of HMC-1 cells

Electron microscopic examination of HMC-1 cells was performed with the methods as previously described (24).

Experimental protocols

To examine the effect of RV14 infection on the production of histamine and cytokines, cells were cultured for 24 h after RV14 or sham infection. To examine the effect of a combination of PMA plus A23187 on the production of histamine and cytokines, cells were further cultured for 1 h for histamine release and 24 h for cytokine release in the presence of PMA plus A23187 or vehicle. Concentrations of PMA and A23187 were 50 and 500 nM in HMC-1 cells and 5 and 50 nM in KU812 cells, except as otherwise described. To examine the effect of IgE plus anti-IgE on the production of histamine and cytokines, cells were pretreated with IgE (1 μg/ml; Athens Research and Technology, Athens, GA) for 4 days. Cells were then cultured for 24 h after RV14 or sham infection and were further cultured for 1 h for histamine release and 24 h for cytokine release in the presence of anti-IgE (1 μg/ml; DAKO, Carpinteria, CA) (25).

Statistical analysis

Results are expressed as means ± SEM. Statistical analysis was performed using a one-way ANOVA, and multiple comparisons were made using Bonferroni's method. Significance was accepted at *p* < 0.05.

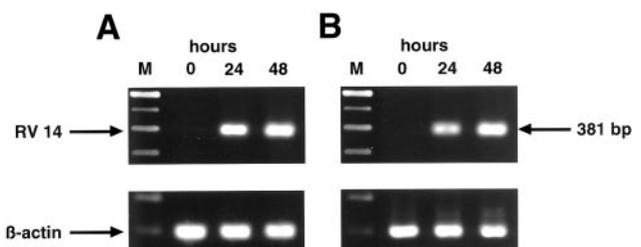


FIGURE 2. Replication of RV RNA from HMC-1 (A) and KU812 (B) cells after RV14 infection detected by reverse transcription. β-actin was used as a housekeeping gene. M represents 100-bp DNA ladder molecular weight markers. Data are representative of three different experiments.

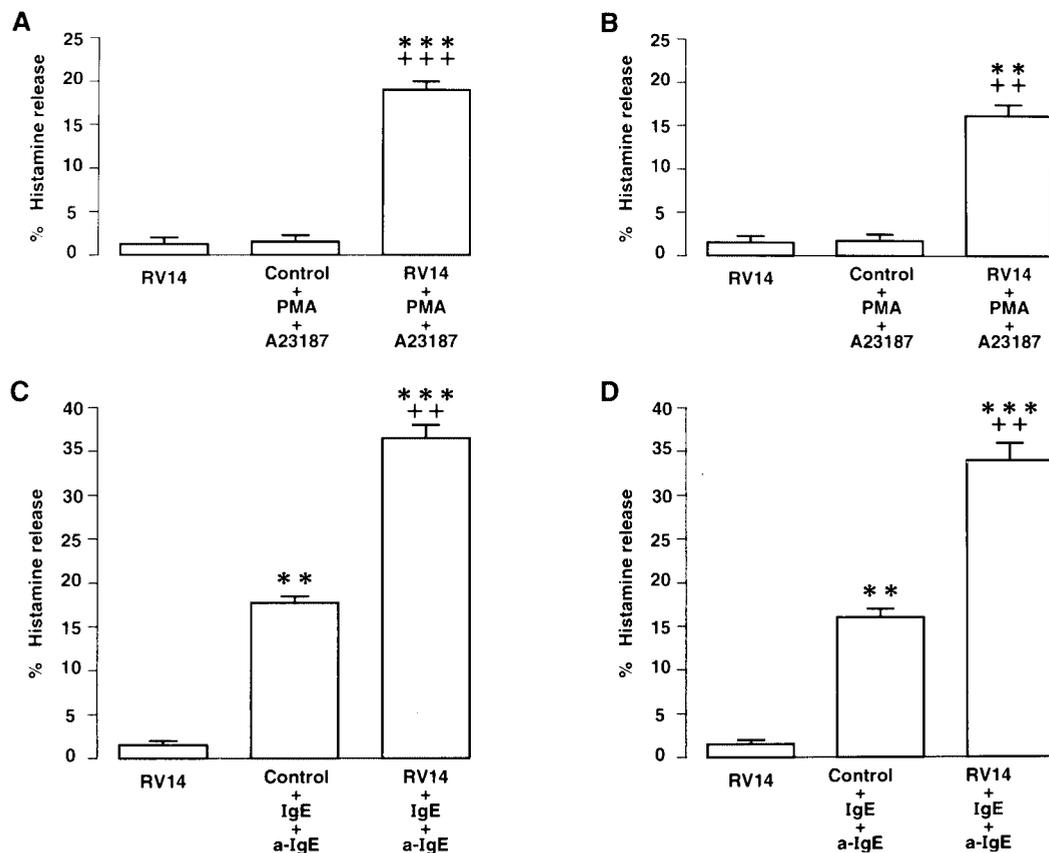


FIGURE 3. Histamine release into supernatants of HMC-1 cells (A and C) and KU812 cells (B and D) in the presence of PMA plus A23187 (PMA + A23187; A and B) or IgE plus anti-IgE (IgE + a-IgE; C and D) after RV14 or sham (control) infection. Results are reported as means \pm SEM from seven samples. Significant differences from RV14 infection alone are indicated by ** ($p < 0.01$) and *** ($p < 0.001$). Significant differences from stimulation with PMA plus A23187 alone or IgE plus anti-IgE alone are indicated by ++ ($p < 0.01$) and +++ ($p < 0.001$).

Results

RV infection of HMC-1 cells and KU812 cells

Exposing a human mast cell line (HMC-1) and a human basophilic cell line (KU812) to RV14 (10^4 TCID₅₀ U/ml) consistently led to infection. Collection of culture medium at 1 h after viral exposure revealed no detectable virus in both cells (Fig. 1). RV14 content in culture medium increased with time between 1 and 24 h after infection, and culture medium collected during 24–48 h contained

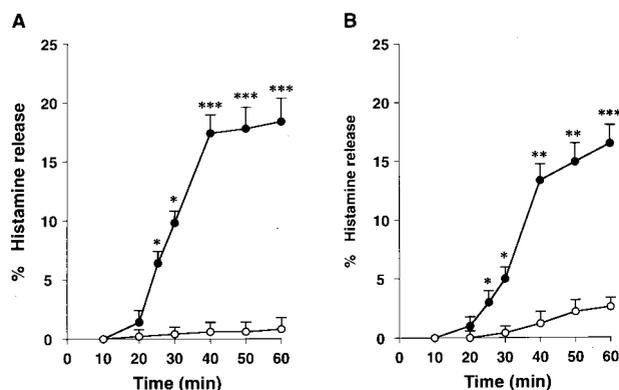


FIGURE 4. Time response effects of PMA plus A23187 on histamine release in HMC-1 cells (A) and KU812 cells (B) 24 h after RV14 (●) or sham (○) infection. Results are reported as means \pm SEM from nine samples. Significant differences from sham infection are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$), respectively.

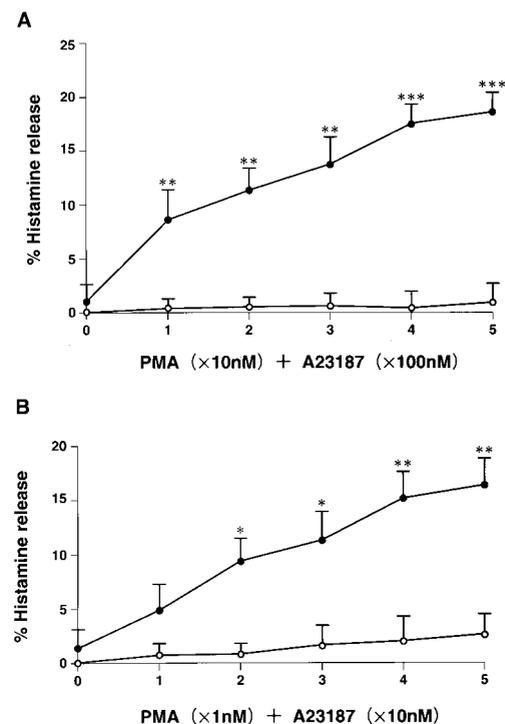


FIGURE 5. Concentration-response effects of PMA plus A23187 (PMA + A23187) on histamine release in HMC-1 cells (A) and KU812 cells (B) 24 h after RV14 (●) or sham infection (○). Results are reported as means \pm SEM. Significant differences from sham infection are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$), respectively.

significant levels of RV14 in both cells (Fig. 1). Viral titer levels in supernatants increased significantly with time in both cells ($p < 0.05$ in each case by ANOVA).

Detection of viral RNA by PCR

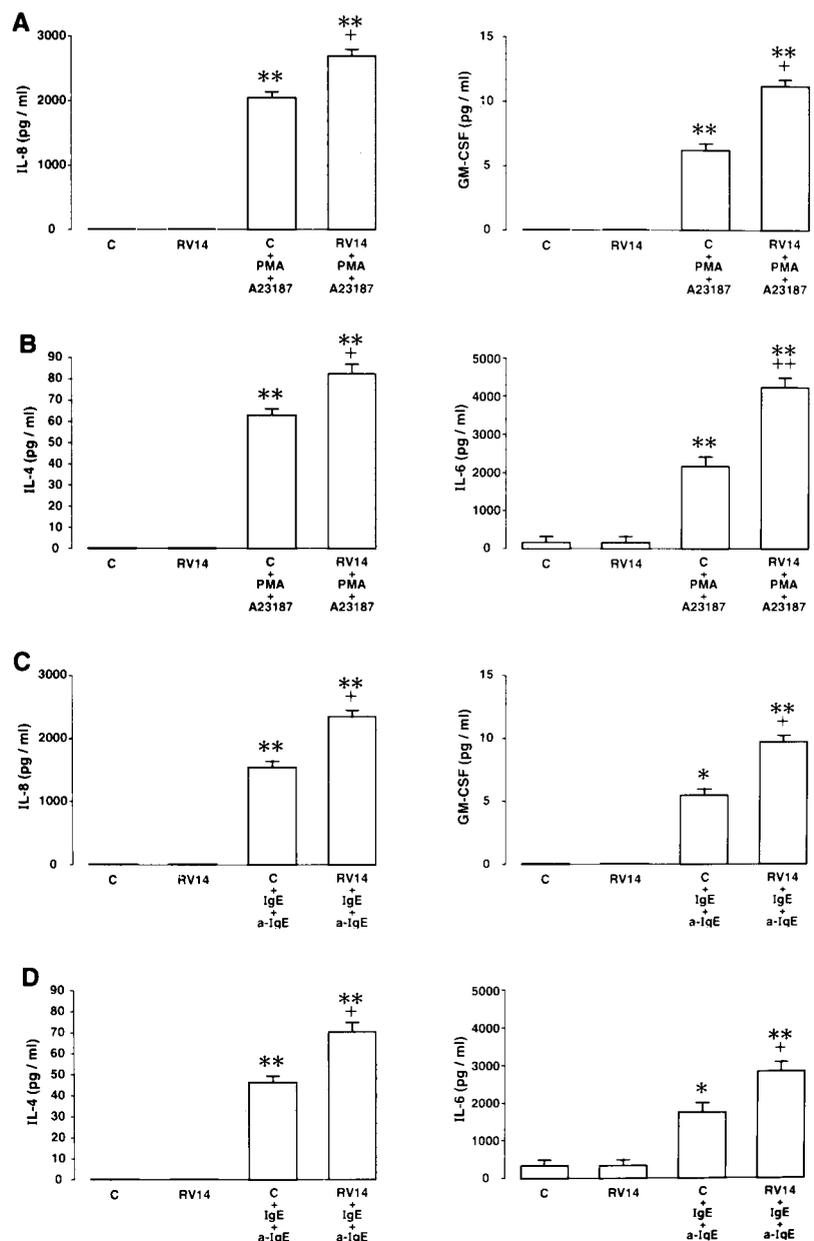
Further evidence of RV14 infection of KU812 and HMC-1 cells and viral replication was provided by PCR analysis (Fig. 2). In each of three experiments, RNA extracted from uninfected cells did not produce any detectable PCR product at 381 bp (0 h), but a pronounced band of viral RNA was observed at 24 and 48 h after infection in both cells (Fig. 2).

Effects of RV infection on histamine release

Total histamine content 24 h after RV14 infection was similar to that of the sham exposure in both HMC-1 cells (27 ± 3 ng/ 10^6 cells in RV infection vs 25 ± 3 ng/ 10^6 cells in sham infection; $p > 0.50$, $n = 7$) and KU812 cells (57 ± 4 ng/ 10^6 cells in RV infection vs 58 ± 5 ng/ 10^6 cells in sham infection; $p > 0.50$, $n = 7$). Fig. 3 shows the percentage of histamine release for 60 min 24 h after

RV14 infection in the presence or absence of stimulation with PMA plus A23187 or IgE plus anti-IgE. RV14 infection alone did not alter the spontaneous histamine release into culture supernatants in either HMC-1 cells or KU812 cells at 24 h after infection ($p > 0.50$, $n = 7$) (Fig. 3). Likewise, PMA plus A23187 alone did not increase histamine release from the cells ($p > 0.50$, $n = 7$) (Fig. 3, A and B). However, PMA plus A23187 significantly increased the histamine release from the cells 24 h after RV14 infection (Fig. 3, A and B). IgE plus anti-IgE alone induced significant increases in histamine release from the cells (Fig. 3, C and D). IgE plus anti-IgE further increased the histamine release from the cells 24 h after RV14 infection (Fig. 3, C and D). The effects of PMA plus A23187 on histamine release 24 h after RV14 infection from both cells was time (Fig. 4) and dose (Fig. 5) dependent. Increased histamine release after stimulation with PMA plus A23187 was observed 25 min after stimulation (Fig. 4). In contrast, PMA plus A23187 failed to increase the histamine release from either HMC-1 cells or KU812 cells 24 h after UV-inactivated RV14 infection (11) (data not shown).

FIGURE 6. A and C, Release of IL-8 and GM-CSF into supernatants of HMC-1 cells in the presence of PMA plus A23187 (PMA + A23187; A), IgE plus anti-IgE (IgE + a-IgE; C) or vehicle after RV14 or sham infection (control). B and D, Release of IL-4 and IL-6 into supernatants of KU812 cells in the presence of PMA plus A23187 (PMA + A23187; B), IgE plus anti-IgE (IgE + a-IgE; D) or vehicle after RV14 or sham infection (control). Results are reported as means \pm SEM from seven samples. Significant differences from sham infection alone (control) and stimulation with PMA plus A23187 alone or with IgE plus anti-IgE alone are indicated by * ($p < 0.05$), ** ($p < 0.01$), + ($p < 0.05$), and ++ ($p < 0.01$), respectively.



Effects of RV infection on cytokine production

Basal secretion was negligible with IL-8 and GM-CSF in HMC-1 cells and IL-4 in KU812 cells, or low with IL-6 in KU812 cells. RV14 infection alone did not alter the production of IL-8 and GM-CSF in HMC-1 cells or that of IL-4 and IL-6 in KU812 cells. In contrast, the production of these cytokines increased in response to the treatment with PMA plus A23187 (Fig. 6, A and B) or IgE plus anti-IgE (Fig. 6, C and D). RV14 infection further increased cytokine production in the presence of PMA plus A23187 or IgE plus anti-IgE. The effects of PMA plus A23187 on cytokine production from the cells were dose dependent (Fig. 7). However, the amount of cytokines in the culture supernatants was larger for the first 24 h than during next 24 h (data not shown). In contrast, the cytokine release caused by PMA plus A23187 after UV-inactivated RV14 infection did not differ from that by PMA plus A23187 alone (data not shown). We detected no IL-1 β , IL-3, IL-4, IL-5, IL-6, TNF- α , IFN- γ , or eotaxin in HMC-1 cells and no IL-1 β , IL-3, IL-5, IL-8, TNF- α , GM-CSF, IFN- γ , or eotaxin in KU812 cells in culture supernatants before and after RV14 infection. Likewise, both IL-1 β and TNF- α were not detectable in viral stocks.

ICAM-1 expression in HMC-1 cells and KU812 cells

Both HMC-1 and KU812 cells expressed a significant ICAM-1-specific fluorescence intensity assayed by flow cytometric analysis. However, RV14 infection alone, PMA plus A23187 alone, or a combination of them did not increase the ICAM-1 specific intensity compared with a sham exposure in both cells (Fig. 8).

Effects of Abs to ICAM-1 on RV infection and production of histamine and cytokines

Incubation of HMC-1 cells and KU812 cells with a mouse mAb to ICAM-1 (84H10) completely blocked RV14 infection, as assessed by the absence of detectable viral titers in the supernatants recovered 24 h after RV14 exposure (5.8 ± 0.5 log TCID₅₀ U/ml in control and 0.0 ± 0.0 log TCID₅₀ U/ml in 84H10 in HMC-1 cells; 4.1 ± 0.2 log TCID₅₀ U/ml in control and 0.0 ± 0.0 log TCID₅₀ U/ml in 84H10 in KU812 cells). Likewise, 84H10 significantly inhibited increases in histamine release and cytokine production induced by PMA plus A23187 after RV14 infection in both cells, whereas an isotype-matched IgG1 control mAb failed to alter them (Fig. 9) as well as viral titers in the supernatants 24 h after RV infection in HMC-1 cells (5.9 ± 0.5 log TCID₅₀ U/ml; $p > 0.50$, $n = 7$) and KU812 cells (4.2 ± 0.2 log TCID₅₀ U/ml; $p > 0.50$, $n = 7$).

Effects of RV infection on [Ca²⁺]_i

The fluorescence intensity caused by [Ca²⁺]_i in both HMC-1 and KU812 cells did not change 2 h after RV14 infection (Fig. 10, A and C). In contrast, both cells 6 h after RV14 infection were shown to induce weak but significant increases in fluorescence intensity caused by the increased number of cells with enhanced [Ca²⁺]_i, compared with a sham exposure (Fig. 10, A and C). The fluorescence intensity then returned to baseline levels 24 h after RV14 infection (Fig. 10, A and C). Likewise, the fluorescence intensity increased at 10 min, 30 min in HMC-1 and KU812 cells and at 60 min in HMC-1 cells after stimulation with PMA plus A23187, and

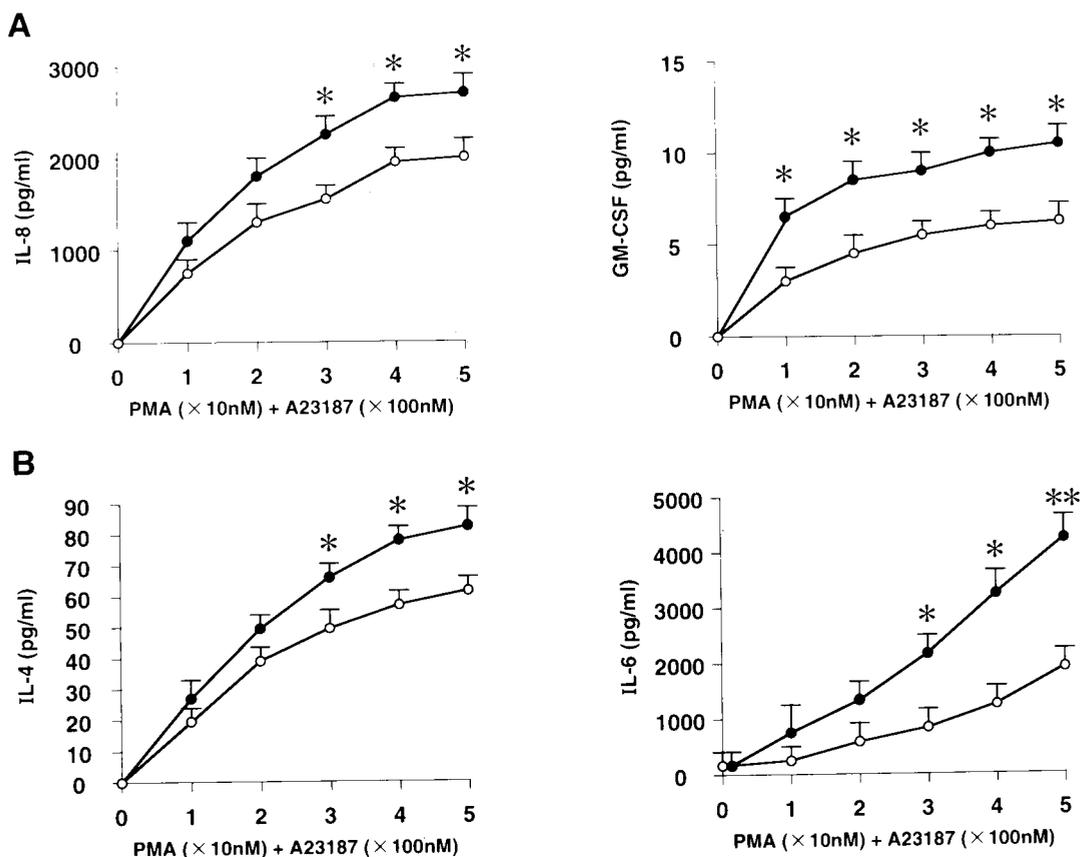


FIGURE 7. Concentration response effects of PMA plus A23187 (PMA + A23187) on cytokine production in HMC-1 cells (A) and KU812 cells (B) after RV14 (●) or sham (○) infection. Results are reported as means \pm SEM. Significant differences from sham infection are indicated by * ($p < 0.05$) and ** ($p < 0.01$), respectively.

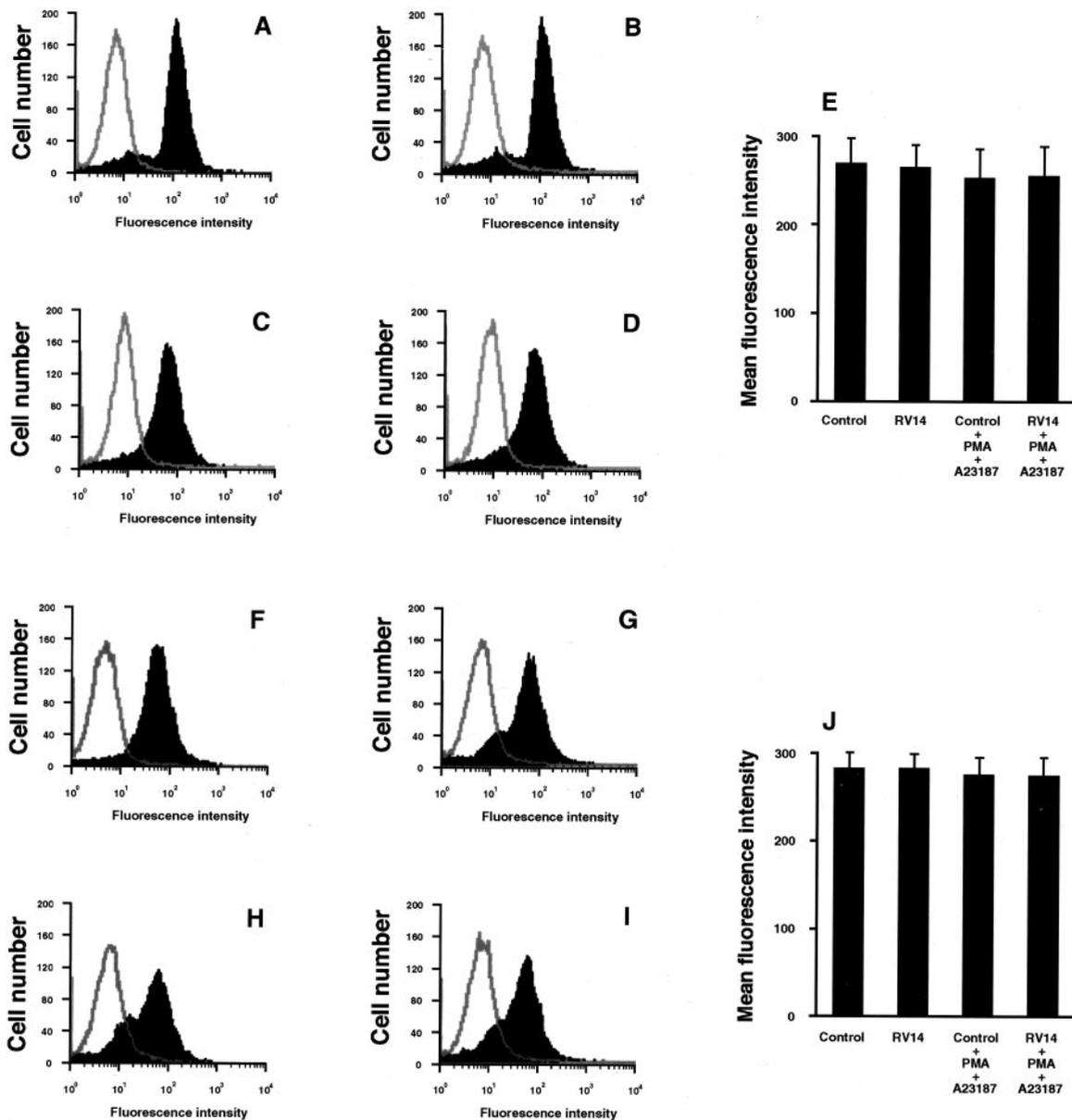


FIGURE 8. Flow cytometry analysis demonstrating the ICAM-1 expression of HMC-1 cells (A–D) and KU812 cells (F–J) in the medium with either PMA plus A23187 (PMA + A23187; B, D, G, and I) or vehicle (A, C, F, and H) after RV14 (C, D, H, and I) or sham (A, B, F, and G) infection. Also shown are effects of RV14 infection (RV14) and PMA plus A23187 (PMA + A23187) on ICAM-1 fluorescence intensity in HMC-1 cells (E) or KU812 cells (J). Results are reported as means \pm SEM from seven samples.

then returned to baseline levels 6 h after stimulation with PMA plus A23187. The fluorescence intensity further increased after stimulation with PMA plus A23187 in both cells infected with RV14 (Fig. 10, B and D).

NF- κ B DNA binding activity in HMC-1 and KU812 cells

Nuclear extracts from HMC-1 and KU812 cells with RV14 or sham infection contained activated NF- κ B, as demonstrated by the presence of a complex consisting of protein bound to a DNA fragment carrying the NF- κ B (Fig. 11). The baseline intensity of NF- κ B binding activity was constant, and increased activation of NF- κ B was present in cells treated with PMA plus A23187 for 2 h, and 0.5 h and 24 h after RV14 infection (Fig. 11). The intensity of NF- κ B binding activity was further increased in the cells treated with PMA and A23187 24 h after RV14 infection (Fig. 11). Specificity of the NF- κ B binding was confirmed by supershift EMSA,

in which Abs to the p50 or p65 subunit of NF- κ B ablated NF- κ B bands (data not shown). The supershifting of the NF- κ B band with the Ab to the p50 or p65 subunit of NF- κ B was constantly observed at any time of the cell culture. However, the supershifting of the NF- κ B band was not observed with Ab to p52, c-Rel, Rel B, or preimmune antiserum (data not shown).

Electron microscopic examination of HMC-1 cells

Most granule contents contained a flocculent material, and there is a central dense condensate in some of the granules in the HMC-1 cells. The structure of whorls or scrolls was observed only very rarely (Fig. 12).

Discussion

In this study we have shown that both human mast cell line HMC-1 cells (16) and human early basophilic leukocyte cell line

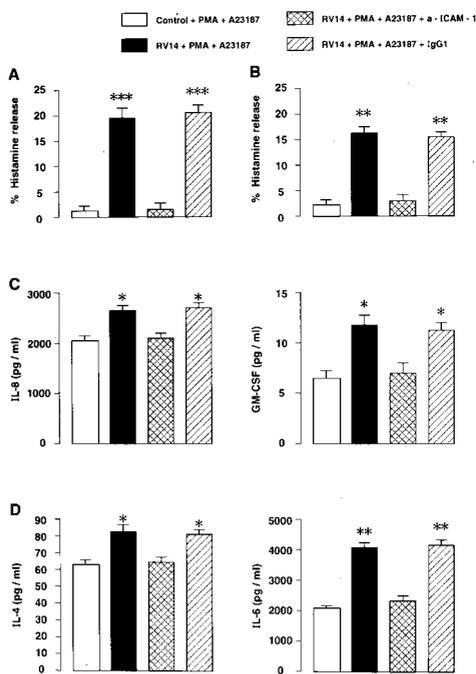


FIGURE 9. Release of histamine (A and B) and cytokines (C and D) into supernatants of HMC-1 (A and C) and KU812 cells (B and D) in the presence of a mAb to ICAM-1 (84H10, a-ICAM-1), mouse purified IgG₁ mAb, or absence of any Ab after RV14 or sham infection (control). Results are reported as means \pm SEM from seven samples. Significant differences from PMA plus A23187 alone (PMA + A23187) are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$), respectively.

KU812 cells (17) can be infected by RV14, a major group RV, and RV14 infection primes the production of IL-4, IL-6, IL-8, GM-CSF, and histamine in response to PMA plus A23187 or IgE plus anti-IgE. These conclusions are based on the observations that RV14 titers of culture supernatants and RV RNA in the infected cells increased with time in both cells. RV14 infection alone failed to induce the production of cytokines and histamine in these cells. However, RV14 infection augmented the production of histamine and cytokines in both cells after stimulation with PMA plus A23187 or IgE plus anti-IgE. A mAb to ICAM-1, a receptor for major RV, inhibited RV14 titers and production of histamine and cytokines in the supernatants.

Although an increase in the production of IL-4 and GM-CSF after RV14 infection was subtle, there was a large production of IL-6 and IL-8 in HMC-1 and KU812 cells after stimulation with PMA plus A23187 as previously reported (14) and after stimulation with IgE plus anti-IgE, and RV14 infection further increased cytokine production. However, the immaturity of HMC-1 cells as demonstrated in granule structure with flocculent material (24, 26) in the electron micrographs might relate to the absence of TNF- α production in this study, although mast cells secrete TNF- α (8, 12). IL-6 induces Ab production in B cells and T cell activation and differentiation (13). RV infection causes infiltration of neutrophils, lymphocytes, and eosinophils in nasal (27) and bronchial mucosa. IL-8 stimulates neutrophils to cause chemotaxis and production of reactive oxygen metabolites (28). Therefore, proinflammatory cytokines released from mast cells and basophils may also relate to the airway inflammation caused by RV infection (7).

The specificity of the infection process for HMC-1 cells and KU812 cells by RV14 was confirmed by demonstrating that an Ab to ICAM-1 completely blocked RV14 infection in HMC-1 and KU812 cells, but an isotype-matched IgG1 mAb did not inhibit it.

Furthermore, an Ab to ICAM-1 inhibited the production of cytokines and histamine in the cells. HMC-1 and KU812 cells expressed ICAM-1 protein as reported previously (29). Therefore, RV14 can infect both cells via binding to ICAM-1 on the cell surface.

The precise mechanisms of the priming effects of RV14 infection on the release of histamine and cytokines from HMC-1 and KU812 cells are uncertain. However, ionomycin and Ag stimulation induce histamine and cytokine production in murine mast cell line and rat basophilic leukemia cells via an activation of protein kinase C and increases in $[Ca^{2+}]_i$ (30, 31). Virus infection increases protein kinase C activity and $[Ca^{2+}]_i$ in a variety of cells, including polymorphonuclear leukocytes (22, 32). In the present study, RV14 infection increased $[Ca^{2+}]_i$ in HMC-1 and KU812 cells 6 h after RV14 infection. The time course of changes in $[Ca^{2+}]_i$ in the cells was similar to that in human fibroblasts by poliovirus infection (22). However, the increased $[Ca^{2+}]_i$ in HMC-1 and KU812 cells after RV14 infection was 10% of control values and smaller than that in human fibroblasts after poliovirus infection (22). Furthermore, $[Ca^{2+}]_i$ in HMC-1 and KU812 cells returned to baseline levels 24 h after RV14. Therefore, the small degree and short duration of increases in $[Ca^{2+}]_i$ in HMC-1 and KU812 cells by RV14 infection alone might not be enough to induce histamine release and cytokine production. In contrast, PMA plus A23187 caused significant and prolonged increases in $[Ca^{2+}]_i$ in HMC-1 and KU812 cells, and RV14 further augmented increases in $[Ca^{2+}]_i$ in these cells. Therefore, the increased $[Ca^{2+}]_i$ by PMA plus A23187 after RV14 infection might be mechanisms responsible for enhanced histamine release and cytokine production.

The precise mechanisms are uncertain why RV14 infection alone did not induce cytokine production in HMC-1 cells and KU812 cells, although RV14 infection alone increased NF- κ B activation, which relates to cytokine production in various cells (4, 23). However, the degree of NF- κ B activation induced by RV14 infection alone might not be enough to increase the cytokine production. Furthermore, HMC-1 cells might be immature for cytokine production in response to NF- κ B activation after RV infection alone. In contrast, RV14 infection further increased the NF- κ B activation induced by PMA plus A23187 in the cells. Therefore, increased activity of NF- κ B in RV14-infected cells might relate to the priming effect of RV14 infection on the production of cytokine after stimulation with PMA plus A23187.

IFN is suggested to induce histamine release from human basophils infected with various viruses, including influenza type A (19). However, neither HMC-1 cells nor KU812 cells secreted IFN in the present study, suggesting that IFN is not responsible for increases in histamine release after RV14 infection. IFN- γ promotes the release of histamine and cytokines, including TNF- α , in human mast cells (12), but inhibits histamine release from rat intestinal mucosal mast cells (33). Thus, the effect of IFN on the histamine release may differ among the different populations of mast cells.

PMA plus A23187 did not increase ICAM-1 protein expression in either HMC-1 cells or KU812 cells after RV14 infection. Although the effects of RV infection on ICAM-1 expression in HMC-1 cells and KU812 cells have not been studied, IL-4 is reported to induce mRNA and protein of ICAM-1 in HMC-1 cells (29), in which a slight induction of ICAM-1 mRNA was observed 6 h after culture with IL-4, and ICAM-1 mRNA expression became obvious 2 days after culture with IL-4 (29). The absence of IL-4 production in HMC-1 cells and low levels of IL-4 production in KU812 cells may explain the failure of ICAM-1 induction in these cells after RV infection.

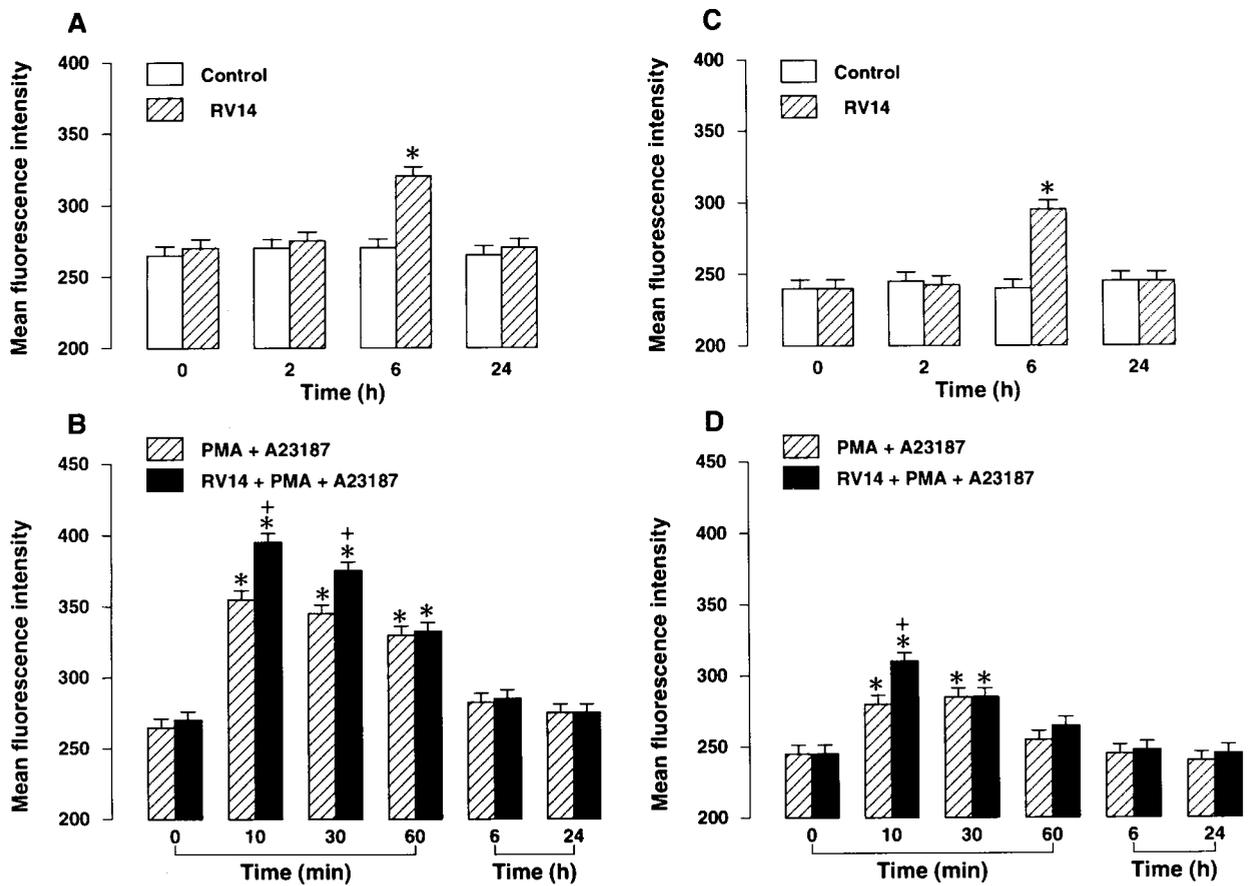


FIGURE 10. A and C, Time course of $[Ca^{2+}]_i$ -specific fluorescence intensity in HMC-1 (A) and KU812 (C) cells before ($t = 0$) and after RV14 infection. Significant difference from medium alone (control) is indicated by * ($p < 0.05$). B and D, Time course of $[Ca^{2+}]_i$ -specific fluorescence intensity before ($t = 0$) and after stimulation with PMA plus A23187 (PMA + A23187) in HMC-1 (B) and KU812 (D) cells 24 h after RV14 or sham infection. Significant differences from medium alone ($t = 0$) are indicated by * ($p < 0.05$). Significant differences from PMA plus A23187 alone (PMA + A23187) are indicated by + ($p < 0.05$).

We demonstrated the first evidence that RV14 infection induces histamine release from mast cell line HMC-1 cells and basophilic cell line KU812 cells (16) after stimulation with PMA plus A23187 or with IgE plus anti-IgE. HMC-1 was established by Butterfield et al. (15) and has been used for studies of mast cell biology (29, 34). PMA plus A23187 alone did not

increase histamine release in either HMC-1 cells or KU812 cells, although A23187 increases histamine release from human basophils (18). Thus, the effects of A23187 or PMA on histamine release may differ among cell types or among culture conditions of the cell line. However, the potency of histamine release from HMC-1 and KU812 cells induced by RV14

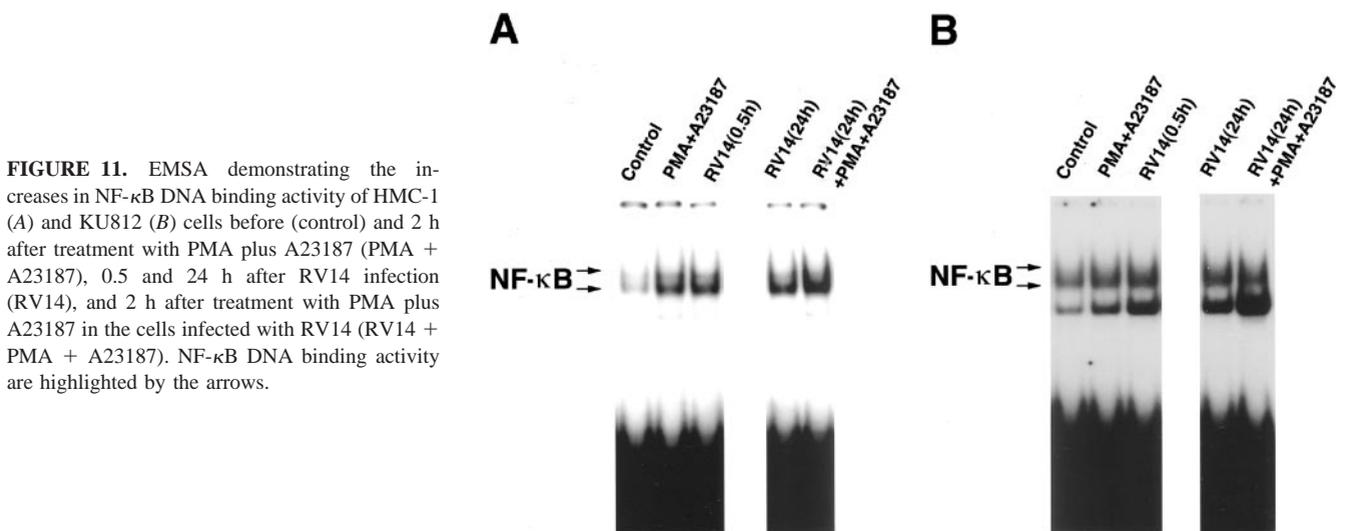


FIGURE 11. EMSA demonstrating the increases in NF- κ B DNA binding activity of HMC-1 (A) and KU812 (B) cells before (control) and 2 h after treatment with PMA plus A23187 (PMA + A23187), 0.5 and 24 h after RV14 infection (RV14), and 2 h after treatment with PMA plus A23187 in the cells infected with RV14 (RV14 + PMA + A23187). NF- κ B DNA binding activity are highlighted by the arrows.

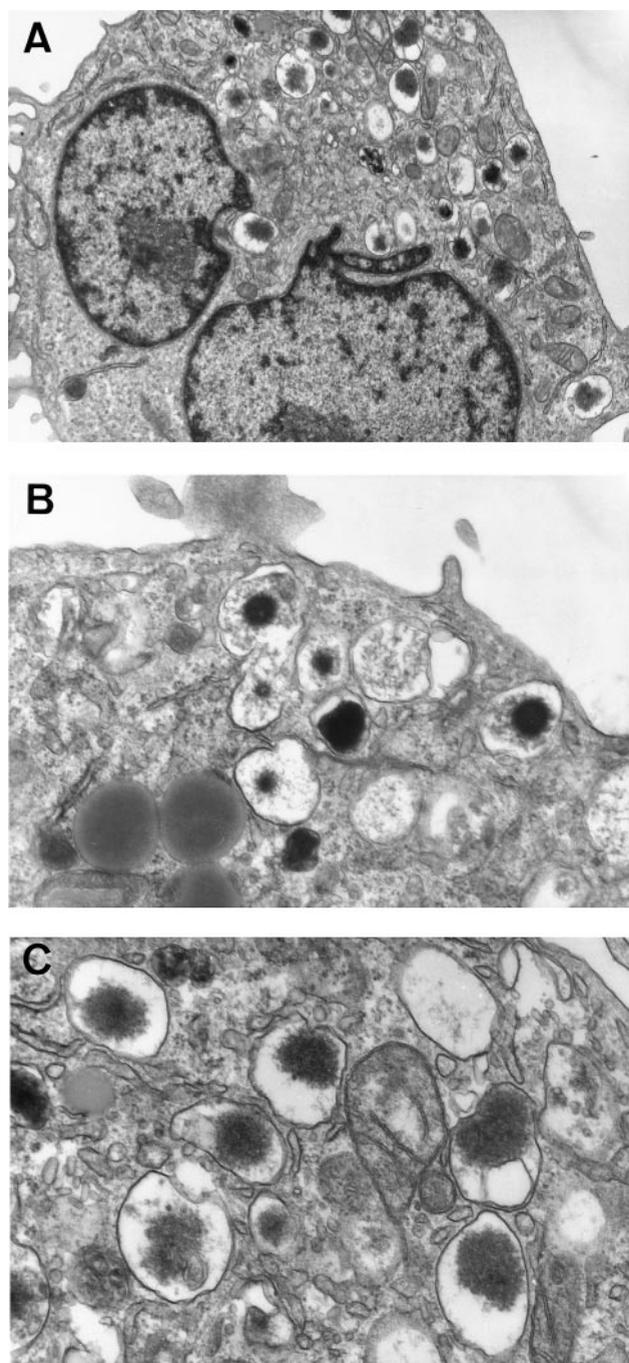


FIGURE 12. Electron microscopy of HMC-1 cells. Magnification: $\times 10,000$ (A), $\times 25,000$ (B), and $\times 30,000$ (C). The granule contents contain a flocculent material, and a central dense condensate can be seen in some of the granules (A and B). The structure of whorls or scrolls are observed only very rarely (C).

infection after stimulation with PMA plus A23187 or with IgE plus anti-IgE is consistent with that induced by virus infections after stimulation with A23187 or anti-IgE in human basophils (18, 19).

Experimental RV infection increases plasma histamine release after Ag bronchoprovocation (6). Likewise, viral respiratory infections increase plasma histamine in children (35) and increase histamine release in nasopharyngeal secretions in patients with wheezing (36). The results of increased histamine release in HMC-1 and KU812 cells after RV14 infection in the present study

are consistent with those reported previously in basophils stimulated with anti-IgE and calcium ionophore after virus infection including RV (18, 19). Because both mast cells and basophils accumulate in the airway of bronchial asthma (37), these cells may be a major source of histamine release, thereby relating to the airway inflammation and exacerbations of bronchial asthma after RV infection.

In summary, we have shown that a major group RV14 infects human mast cell line HMC-1 cells and peripheral blood basophilic cell line KU812 cells. RV14 infection increased histamine release and cytokine production in these cells in response to PMA plus A23187 and IgE plus anti-IgE. Therefore, mast cells and basophils may participate in airway inflammation and exacerbations of bronchial asthma after RV infection.

Acknowledgments

We thank Grant Crittenden for the English correction and Akira Ohmi, Fusako Chiba, and Michiko Okamura for technical assistance.

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