Respiratory syncytial virus infection of primary human mast cells induces the selective production of type I interferons, CXCL10, and CCL4

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Background: Respiratory syncytial virus (RSV) causes severe respiratory tract infections, which might have a role in the development of airway hyperreactivity. Mast cells are important effector cells in allergy, with sentinel cell roles in host defense. However, the role of mast cells in response to RSV infection is unknown.

Objective: Human mast cell responses to RSV were investigated with a view to better understanding the role of mast cells in RSV-induced disease.

Methods: Human cord blood–derived mast cells and the HMC-1 mast cell line were exposed to RSV or UV-inactivated RSV. Viral gene and protein expression were evaluated by using PCR and flow cytometry. The expression of interferon-stimulated genes and selected mediators were evaluated by using quantitative PCR and ELISA.

Results: Human mast cells expressed multiple RSV genes after exposure to RSV, and a small percentage of mast cells supported RSV antigen protein expression. RSV induced mast cells to upregulate production of chemokines, including CCL4, CCL5, and CXCL10, as well as type I interferons, and interferon-stimulated gene expression. However, production of the granulocyte chemoattractants CXCL8 and CCL11 was not induced. Antibody blockade of the type I interferon receptor on human cord blood–derived mast cells reduced the RSV-mediated induction of CXCL10 and CCL4 but not CCL5. Leukotriene C_4 production by mast cells was not enhanced by exposure to RSV.

Conclusion: Despite low levels of infection, human mast cells produce multiple chemokines in response to RSV through mechanisms that include responses to type I interferons. Such mast cell responses might enhance effector cell recruitment during RSV-induced disease. (J Allergy Clin Immunol 2015;136:1346-54.)

Key words: *Mast cells, viral infection, innate immunity, respiratory infection, chemokines, interferons, respiratory syncytial virus*

Respiratory syncytial virus (RSV) is a pneumovirus belonging to the Paramyxoviridae family with a nonsegmented, negative-sense, single-stranded RNA genome.[1] RSV infection is a particular hazard to young children, the elderly, and immunocompromised patients. It is the leading cause of bronchiolitis and pneumonia in children and hospitalization of infants worldwide. RSV is also a major contributor to child mortality in developing countries.[2-5] There is still no effective vaccine for RSV, and reinfection with RSV is common.[5,6] RSV infects mainly the respiratory epithelium, although restricted replication in human and mouse alveolar macrophages,[7] myeloid dendritic cells (DCs), and plasmacytoid dendritic cells (pDC) has been reported,[8-10] resulting in functional changes.[11,12] RSV-associated pathology is characterized by an early neutrophil infiltrate of the peribronchial areas and a mononuclear infiltrate in the interstitium of the lung consisting of monocytes, double-negative $CD3^+$ T cells, and $CD8^+$ T cells.[13] There are also reports of eosinophil degranulation in patients with severe disease.[14,15] Initial T_H2 skewing is associated with prior experimental immunization[16] or occurs following neonatal infection of mice.[13] Effective immunity to RSV is thought to be regulated by a number of mechanisms, including the nature of the primary responding effector cells, the subsequent inflammatory cascade, and the ability of RSV to subvert the function of professional antigen-presenting cells.

It is likely that immune sentinel cells in the airways, including mast cells, play a role in the immune response to RSV. Mast cells are immune effector cells best known for their role in acute allergy and asthma.[17] They are particularly prevalent within the skin, gastrointestinal tract, and airways and are strategically located in the perivasculature and the interstitium of the human lung. Mast cells play a sentinel role in protecting the body from pathogens,

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Abbreviations used
CBMC: Human cord blood–derived mast cell
DC: Dendritic cell
ISG56: Interferon-stimulated gene 56
LTC_4: Leukotriene C_4
MOI: Multiplicity of infection
MX1: Myxovirus resistance gene 1
NK: Natural killer
pDC: Plasmacytoid dendritic cell
qPCR: Quantitative PCR
RSV: Respiratory syncytial virus

as shown in experimental models of bacterial and nematode parasite infection.[18] The ability of mast cells to contribute to host defense includes the recruitment of effector cells through cytokine and chemokine production[19-22] and the initiation of acquired immunity through mobilization of DC responses.[23-26] The ability of mast cells to contribute to host defense against viral infection is less well defined. However, mast cells can be infected by a number of viruses, including HIV, hantavirus, reovirus, dengue virus, and influenza A virus, and have been shown to selectively produce mediators that activate vascular endothelium and recruit immune effector cells.[27-31]

The nature of virus-induced mediator production by mast cells is dependent on the type of stimulation. For example, reovirus infection of human mast cells leads to production of substantial amounts of CXCL8, which enhances the migration of both human neutrophils and natural killer (NK) cells.[21] Through a distinct mechanism, involving CCL3, CCL4, and CCL5, reovirus-infected mast cells will recruit $CD56^+$ T cells.[22] In contrast, human mast cells infected by low doses of dengue virus in the presence of subneutralizing concentrations of anti-dengue antibodies produce little CXCL8 but have enhanced CCL4, CCL5, and CXCL10 responses that could be associated with T-cell subset recruitment.[28] The effect of RSV on human mast cell mediator production is of particular interest given the suggested links between severe RSV-induced disease and the development of childhood wheeze.[32] Very few studies have investigated the role of mast cells in the pathogenesis of RSV-induced disease. However, bronchoalveolar lavage samples obtained from infants hospitalized with RSV had higher levels of mast cell tryptase,[33] which are indicative of mast cell degranulation. Furthermore, infants with RSV-induced bronchiolitis had higher levels of urinary 9α,11β–prostaglandin F_2, a marker of mast cell lipid mediator production.[34] Studies using guinea pig and rat models of RSV infection found an increased number of mast cells in lung tissue samples of animals infected with the virus compared with control animals.[35,36] Mast cells are a major source of mediators associated with bronchoconstriction, such as leukotriene C_4 (LTC_4),[18] and can also produce other mediators found to be increased in the sputum and lavage fluid of patients with RSV infections.[15,18,32,37-39] These findings suggest that mast cell responses to RSV can contribute to the pathogenesis of the disease.

In the current study the ability of RSV to infect primary cultured human mast cells and to induce the production of inflammatory mediators was investigated. RSV treatment of mast cells led to viral gene transcription and upregulation of interferon response genes and selective mast cell type I interferon and chemokine responses, with important implications for early antiviral responses.

METHODS

Cell lines

HMC-1 and KU812 cells were grown in Iscove modified Dulbecco medium (Invitrogen, Burlington, Ontario, Canada) supplemented with 10% FBS (Medicorp, Montreal, Quebec, Canada), 1% L-glutamine (Thermo Fisher Scientific, Nepean, Ontario, Canada), 100 U/mL penicillin G, and 100 μg/mL streptomycin (Invitrogen).

Primary mast cells

Human cord blood–derived mast cells (CBMCs) were derived from umbilical cord blood obtained with permission provided by the Izaak Walter Killam Health Centre research ethics board (Halifax, Nova Scotia, Canada) and cultured according to adaptations of the methods of Saito et al[40] and Radinger et al[41]; they were at least 95% pure as assessed by CD117 staining. Briefly, mononuclear cells from cord blood were cultured in the presence of 100 ng/mL stem cell factor for 6-12 weeks with weekly changes in media and the long lived mast cells that became predominant in these cultures over time were used for experiments. In some cases CBMC cultures were further sorted with a FACSAria II cell sorter (BD Biosciences, San Jose, Calif) to isolate $CD117^{hi}$ mature mast cells. Before activation, CBMCs were cultured overnight in medium devoid of prostaglandin E_2 and with 10 ng/mL stem cell factor. Mast cells produced in this way were on average ($n = 4$) 96.54% positive for CD117 (c-kit), 99.26% positive for mast cell tryptase, and less than 0.2% positive for a variety of other lineage markers including CD3, CD19, CD56, CD11b, CD56, CD86, CD163 and CD209. Previous studies have demonstrated that these types of mast cells express little TLR3 or TLR4 at the protein level, without further cytokine treatments.[42]

RSV propagation and treatment

RSV (Long strain) was propagated in HEp-2 cells in RPMI-1640 medium containing 2.5% heat-inactivated FBS and then harvested when infected cultures showed 80% to 90% cytopathic effect. The HEp-2 cells were lysed in RNAse-free water, returned to physiologic osmolality with PBS (10×, Thermo Fisher Scientific), and finally centrifuged at 2095*g* for 10 minutes. This inoculum routinely contained approximately 1×10^7 plaque-forming units/mL. Where specified, RSV was pretreated with palivizumab (Abbot, Saint-Laurent, Quebec, Canada) or pooled human IgG as a control at 10 μg/mL for 30 minutes at 4°C before addition to cells.

Inoculation conditions

CBMCs or HMC-1 cells were inoculated with RSV, RSV pretreated with palivizumab or human IgG, UV-inactivated RSV, or a medium control for 90 minutes at 4°C. Mast cells were inoculated at a multiplicity of infection (MOI) of 3 to 4 unless otherwise specified. Cells were washed and either analyzed at this time point as a control for experiments assessing mast cell infection or incubated at 37°C in RPMI-1640 with 2.5% FCS (containing 10 ng/mL stem cell factor for CBMCs) and soybean trypsin inhibitor (Sigma-Aldrich, St Louis, Mo) at 100 μg/mL, the latter being included to reduce the proteolytic degradation of mast cell products. A peptidase inhibitor, acivicin (Sigma-Aldrich), was included at 0.5 mmol/L in experiments in which LTC_4 levels were measured. CBMCs were incubated at an initial concentration of 1×10^6 cells/mL, HMC-1 cells at 0.5×10^6 cells/mL. By the 24-hour time point, because the HMC-1 cells have a doubling time of 24 hours in culture and the mature CBMC are no longer dividing, both cell types were at a similar concentration. Where indicated, CBMCs were incubated in the presence of an anti–IFN-α/β receptor antibody (MMHR-2 clone; Calbiotech, Spring Valley, Calif) at a concentration of 5 μg/mL or an isotype control. After incubation, cells, supernatants, and cell lysates were harvested for mediator analysis.

The effect of RSV-infected CBMC supernatants on RSV infection of HEp-2 cells was performed with RSV infection as described above, using a dose which infected close to 20% of the HEp-2 cells. Supernatants from matched RSV- or mock-infected CBMC 24 hour cultures were added during adsorption and maintained throughout the 24 hour post-infection HEp-2 culture.

Plaque assays

Aliquots of clarified virus or CBMC cell lysates from RSV-treated cells were serially diluted and inoculated onto 80% to 90% confluent HEp-2 cell monolayers. Plaque assays were performed as previously described.[43,44]

Fluorescence-activated cell sorting staining and analysis

For CD117/c-Kit staining, CBMCs were incubated with allophycocyanin-conjugated anti-human CD117 mAb or mouse IgG_1 (eBioscience, San Diego, Calif) at a concentration of 1 μg/mL. For intracellular RSV antigen staining, cells were fixed with 1% paraformaldehyde, permeabilized in 0.2% saponin (Sigma-Aldrich), and incubated with biotin-conjugated goat anti-RSV serum (Meridian Life Sciences, Memphis, Tenn) at 5 μg/mL. Biotin-labeled goat IgG (Jackson ImmunoResearch, West Grove, Pa) was used as a control. Cells were then incubated with streptavidin-conjugated PE (eBioscience) and fixed in 1% paraformaldehyde. Samples were acquired with a FACSCalibur (BD Biosciences). Intracellular RSV antigen expression was determined by using 0-hour readings (immediately after RSV adsorption and washing) as a control, and data were analyzed with FCS Express 3 software (Denovo Software, Los Angeles, Calif).

Quantitative PCR

Total RNA was extracted with the RNeasy Plus Mini Kit (Qiagen, Mississauga, Ontario, Canada). Genomic DNA was depleted, and cDNA was generated with the QuantiTect Reverse Transcription Kit (Qiagen). Commercial primer pairs (Qiagen) for glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)*, hypoxanthine phosphoribosyltransferase 1 *(HPRT1)*, *CCL4*, *CCL5*, *CXCL10*, *IFNA1*, *IFNB*, interferon-stimulated gene 56 *(ISG56)*, and myxovirus resistance gene 1 *(MX1 [MxA])* were used to assess the relative expression of genes. RSV-specific primers for *NS1*, *N*, and *F* (Sigma) were as described by Boukhvalova et al.[44] The QuantiFast SYBR Green PCR Kit (Qiagen) was used to perform duplicate quantitative PCR (qPCR) reactions in the MX-3000P QPCR System (Stratagene, La Jolla, Calif). Data were analyzed with MxPro Software (Stratagene), and the fold induction of gene expression was calculated by using the Pfaffl method,[45] or when appropriate, they were normalized to *HPRT1* and *GAPDH* and expressed as 2 to the power of the difference between the threshold cycles for amplification of the reference gene and target gene.

Mediator analysis

ELISAs were performed to measure the supernatant concentrations of human CCL4 (R&D Systems, Minneapolis, Minn), CXCL10 (BD PharMingen), and CCL11 (PeproTech, Rocky Hills, NJ). The CCL5 ELISA used a rabbit anti-human CCL5 antibody (Endogen, Woburn, Mass) with a goat anti-human secondary antibody (R&D Systems). LTC_4 levels were measured according to a modification of the protocol from Volland et al.[46] A commercial high-sensitivity amplification system (Invitrogen) was used for all ELISAs. For detection of IFN-α2 and IFN-β in supernatants and selected CXCL10 measurements, Luminex assays were used (eBioscience and Bio-Rad Laboratories, Hercules, Calif).

Statistical analysis

HMC-1 cell line data were analyzed by using repeated-measures 1-way ANOVA, followed by the Dunnett multiple comparison test. In experiments with CBMCs, data were either log transformed and analyzed with the paired *t* test or, for multiple comparisons, analyzed with repeated-measures 1-way ANOVA, followed by the Sidak comparison for selected pairs. In some cases (as stated), in view of nonnormal data distribution, data were directly analyzed by using the Friedman test, followed by the Dunn selected pairs comparison. *P* values of less than .05 were considered significant. All statistical analyses were performed with Prism 6 software (GraphPad Software, La Jolla, Calif).

RESULTS

Human mast cells support RSV antigen expression

The intracellular expression of RSV proteins was quantified by means of flow cytometry in CBMCs inoculated with RSV or UV-inactivated RSV or treated with a medium control to investigate the effect of RSV exposure on human mast cells. Maximum viral antigen expression was detected in response to live but not UV-inactivated virus in a small proportion of $CD117^{hi}$ cells (ie, mature mast cells) at 24 hours after infection (median, 0.7%; interquartile range, 0.5% to 1.2% in RSV-infected cells vs 0% in UV-RSV treated cells; n = 12 donors; Fig 1, *A*). Similar levels of viral protein expression were observed after 48 hours (n = 3), but by 72 hours, no viral protein expression was detected. CBMCs maintained similar viabilities in all groups. In addition, the expression of 3 representative RSV genes, *NS1*, *N*, and *F*, was detected by using qPCR at 24 hours in CBMCs exposed to RSV (Fig 1, *C*). Pretreatment of RSV with palivizumab reduced the percentage of cells expressing RSV antigen by 49% ± 14.9% (mean ± SEM, n = 8 donors).

The human mast cell line HMC-1 was inoculated with RSV at increasing MOI levels (0.6-0.8, 1.5-2, 3-4, and 6-8), incubated for 24 hours, and stained to detect intracellular RSV antigen expression to confirm that these responses were not limited to CBMCs. There was increased RSV antigen expression by cells treated with live RSV compared with those treated with UV-inactivated RSV ($P < .01$; Fig 1, *B*) at higher MOI levels. PCR analysis confirmed the expression of RSV genes after HMC-1 infection (data not shown).

Plaque assays were performed to determine whether mast cells were productively infected with RSV. CBMC cell lysates were harvested from 5 different donors after 24 and 48 hours of RSV infection and compared with RSV-infected HEp-2 stock in plaque assays. No plaques were detected from RSV-inoculated CBMCs, whereas plaques were observed from HEp-2 cells after 48 hours, indicating that treatment of CBMCs with RSV does not lead to substantial infectious virus formation.

The possibility that mast cell products acted directly to inhibit the ability of RSV to infect cells was also examined experimentally. The HEp-2 epithelial cell line was infected with RSV in the presence or absence of supernatants from CBMC that had been infected with RSV for 24 hours or mock-infected mast cell supernatants. Our results demonstrate that CBMC supernatants had no significant direct impact on the ability of RSV to infect these epithelial cells under our experimental conditions. Specifically, mock-infected HEp-2 cells were <1% RSV antigen positive, RSV infected HEp-2 cells were a mean of 19.7 ± 0.5% RSV positive, HEp-2 cells infected with RSV in the presence of supernatants from mock-infected CBMC were 21.7 ± 5.6% positive, while HEp-2 cells infected with RSV in the presence of supernatants from RSV infected CBMC were 18.7 ± 3.7% positive (n = 3).

CBMCs produce selective chemokines in response to RSV

CCL4, CCL5, CCL11, CXCL10, and CXCL8 levels are increased in nasal and bronchoalveolar lavage samples of children hospitalized with severe RSV-induced bronchiolitis.[15,32,38,39,47-49] Mast cell production of these cytokines in response to RSV was examined. CBMCs were stimulated for 24 hours with medium, RSV, UV-inactivated RSV, or RSV pretreated with either palivizumab or human IgG control. Protein levels of chemokines were quantified in cell-free supernatants 24 and 48 hours after RSV treatment. CBMCs inoculated with RSV produced significantly higher amounts of CXCL10 and CCL4 at 24 hours compared with medium control–treated

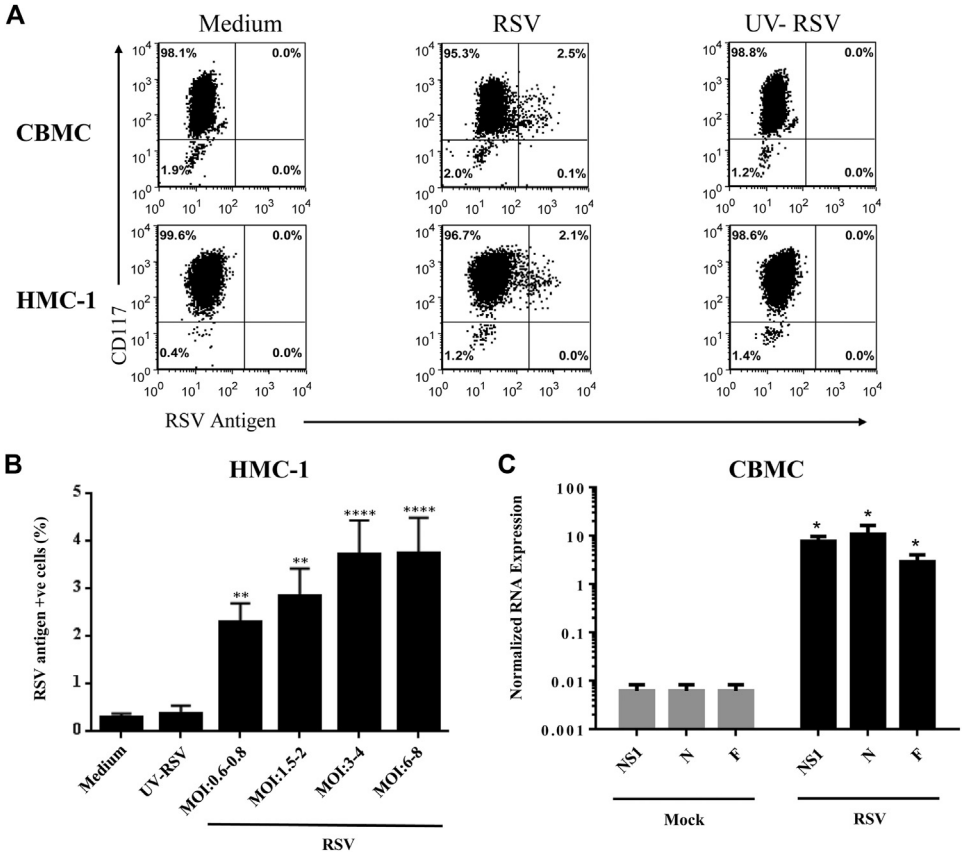

FIG 1. RSV infection of primary human mast cells (CBMCs) and HMC-1 cells. **A,** Intracellular RSV antigen expression in CBMCs (representative of n = 13 donors) and in HMC-1 cells 24 hours after infection. **B,** RSV antigen expression 24 hours after infection in HMC-1 cells at different MOIs (n = 4, mean ± SEM). **C,** Induction of the RSV genes *NS1, N,* and *F* in RSV-infected CBMCs compared with that seen in mock-treated control cells 24 hours after infection (n = 7 CBMC donors, mean ± SEM). **P* < .05, ***P* < .01, and *****P* < .0001.

(*P* < .01 for both) and UV-RSV–treated (*P* < .05 and *P* < .01, respectively) cells (Fig 2, *A*). This response was highly selective, with neither CCL11 (Fig 2, *A*) nor CXCL8 being produced at significant levels (data not shown). The amounts of CCL5 produced were much lower than those of CCL4 and CXCL10 in response to RSV. However, CCL5 production from CBMCs was still increased in response to RSV compared with medium control– or UV-inactivated virus–treated cells (*P* < .05; Fig 2, *A*). Chemokine levels detected in CBMC supernatants at 48 hours were diminished compared with those at the 24-hour time point, but a similar trend in chemokine production was observed. It is possible that the chemokines might have been degraded by mast cell proteases. RSV pretreatment with palivizumab showed a trend toward lower CXCL10, CCL4, and CCL5 responses compared with those in infected cells or control IgG-treated RSV-infected cells (Fig 2, *A*).

A time-course analysis for selected chemokines was performed to delineate the kinetics of the chemokine response by CBMCs. CXCL10 and CCL4 levels started to increase sharply after 6 hours and continued to increase until 24 hours (data not shown). In addition, induction of chemokine mRNA relative to that seen in control-treated cells was measured by using qPCR for CXCL10, CCL4, and CCL5. Levels of these mRNAs were significantly increased for the RSV group compared with the UV-RSV group (*P* < .01; Fig 2, *B*).

To further confirm these results, HMC-1 cells were treated with various MOIs of RSV or controls, and the supernatant levels of CXCL10, CCL4, and CCL5 at 24 hours after infection were quantified by using ELISA. HMC-1 cells produced CCL4, CCL5, and CXCL10 in a dose-dependent manner after RSV inoculation when compared with levels seen in control cells (Fig 3).

CBMCs do not produce LTC_4 in response to RSV

Mast cells produced substantial amounts of LTC_4 after activation with calcium ionophore at 30 minutes (median, 8,677 pg/mL; interquartile range, 4,682-17,265; n = 4 donors). However, no significant LTC_4 response was observed by CBMCs from the same donors treated with RSV either at 30 minutes or later time points (4-6 hours, 12 hours, and 18-24 hours).

RSV-induced mast cell production of selected chemokines depends on early type I interferons

Type I interferons mediate their antiviral effector actions through the activation of a group of interferon response genes, such as *ISG56 (IFIT1)* and *MX1 (MxA)*.[50] To elucidate a potential mechanism for regulation of chemokine production in mast cells exposed to RSV, we first investigated the type I interferon responses in CBMCs 24 hours after infection. There was higher

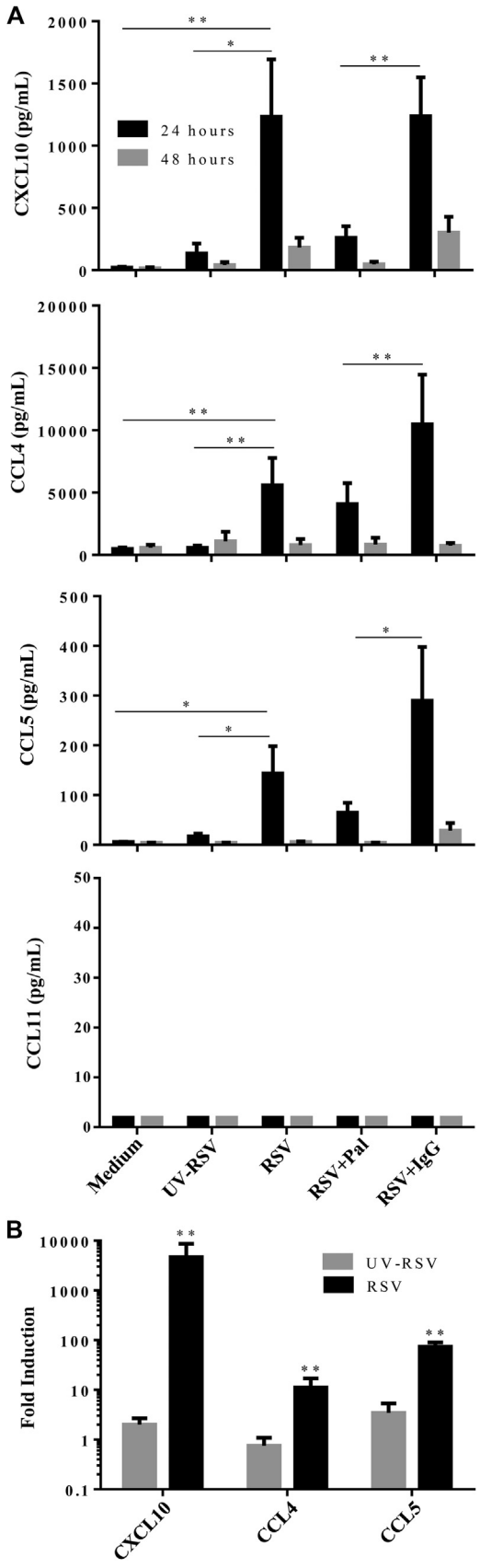

FIG 2. Chemokine production from CBMCs 24 and 48 hours after RSV infection. **A,** Levels of chemokines 24 hours and 48 hours after RSV infection with and without blockade of infection with palivizumab (*Pal*; for CXCL10 24 and 48 hours, n = 9; CCL4 24 hours, n = 12; CCL4 48 hours, n = 5; CCL5 24 hours, n = 10; CCL5 48 hours, n = 4; CCL11 n = 2; mean ± SEM). **B,** CXCL10, CCL4, and CCL5 gene upregulation in RSV-treated CBMCs and UV-inactivated virus–treated CBMCs compared with that seen in medium-treated CBMCs 24 hours after infection (n = 5 donors, mean ± SEM). **P* < .05 and ***P* < .01.

expression of *IFNA1* (*P* < .05), *IFNB*, *ISG56*, and *MX1* mRNA (*P* < .01) in CBMCs inoculated with RSV than in UV-RSV–treated cells (Fig 4, *A*). Supernatants from CBMCs treated with RSV had consistently higher levels of IFN-α2 (*P* < .05) and IFN-β compared with medium-treated or UV-inactivated virus-treated cells (Fig 4, *B*). Mast cells did not produce IFN-γ after RSV stimulation at 24 or 48 hours (Fig 4, *C*).

To evaluate the role of type I interferon responses by mast cells in their chemokine profile after RSV exposure, we blocked the type I interferon receptor after virus adsorption and then collected supernatants for chemokine assays. The efficacy of type I interferon receptor blockade was confirmed in the KU812 cell line stimulated with IFN-α2 (see Fig E1 in this article's Online Repository at www.jacionline.org). CBMCs activated with RSV produced lower CXCL10 and CCL4 protein levels when treated with type I interferon blocking antibody compared with when treated with an isotype control antibody (*P* < .01, Fig 5). In contrast, CCL5 production by mast cells in response to RSV was not affected (Fig 5). No significant inhibitory effect on viral antigen expression was noted with type I interferon receptor blockade.

DISCUSSION

This study demonstrates that human mast cells respond with substantial and selective type I interferon and chemokine production after direct exposure to RSV, despite limited classical viral infection. These findings implicate mast cells in early monocyte and T-cell recruitment after RSV infection. Although RSV genes were transcribed and a low level of RSV antigen expression was detected in CBMCs, there was no detectable production of mature progeny virus from mast cells. This suggests that mast cells are capable of mobilizing aspects of an antiviral response without significantly furthering viral propagation.

The production of mediators after RSV infection of mast cells was highly selective and dependent on exposure to active virus. This suggests that either fusion,[51] uptake of active virus, or some degree of viral activity was required to directly or indirectly initiate these responses. There are several mechanisms whereby viral infection can induce expression of interferons and chemokines. Many of these rely on direct interaction of viral RNA or DNA with sensors within the cell, such as retinoic acid–inducible gene 1, melanoma differentiation-associated protein 5, and Toll-like receptors 3, 7, and 9.[52] UV-inactivated virus was not an effective inducer of mediator production from mast cells, suggesting that viral binding alone is not sufficient to induce the observed mediator responses.

The large amount of chemokines produced by human mast cells after limited RSV infection, together with the requirement of active virus for chemokine induction, suggests an indirect chemokine induction mechanism. We demonstrate not only that RSV infection induces mast cells to produce type I interferons (Fig 4) but also that type I interferon receptor blockade on CBMCs

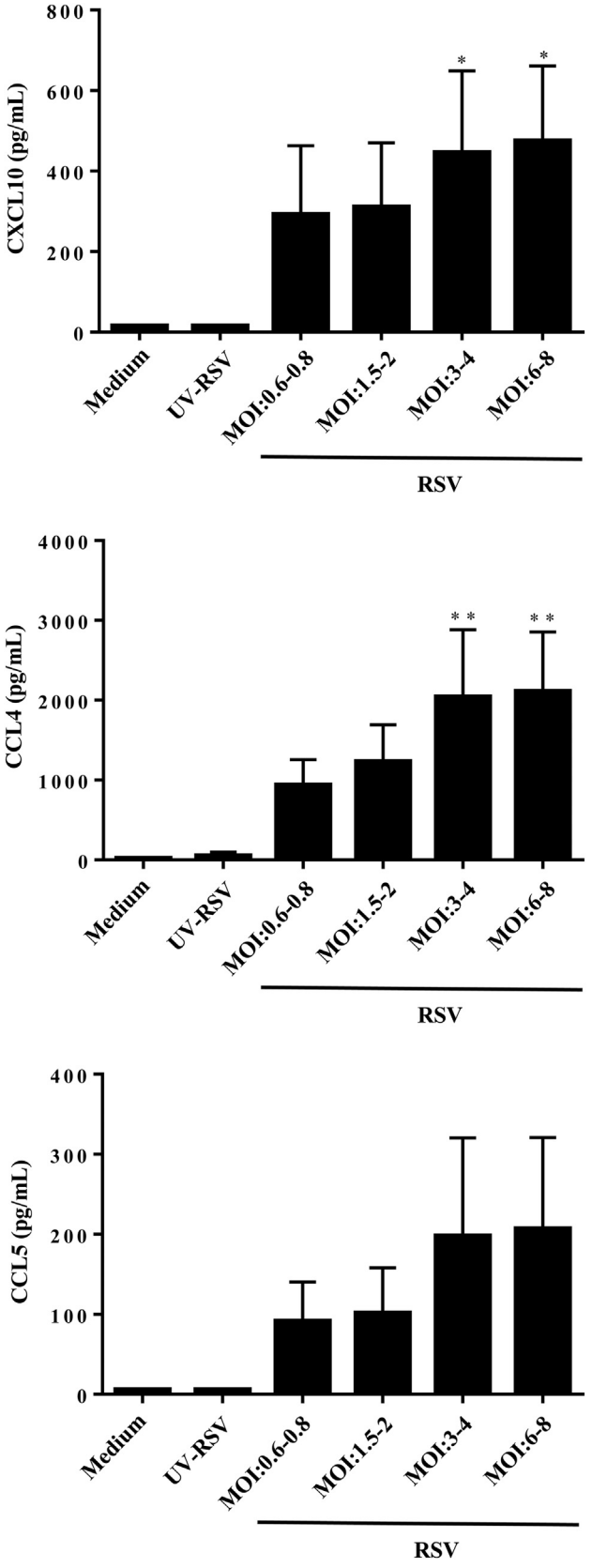

FIG 3. Dose-dependent chemokine production from HMC-1 cells at 24 hours after infection with varying MOIs of RSV (n = 4, mean ± SEM). *$P < .05$ and **$P < .01$.

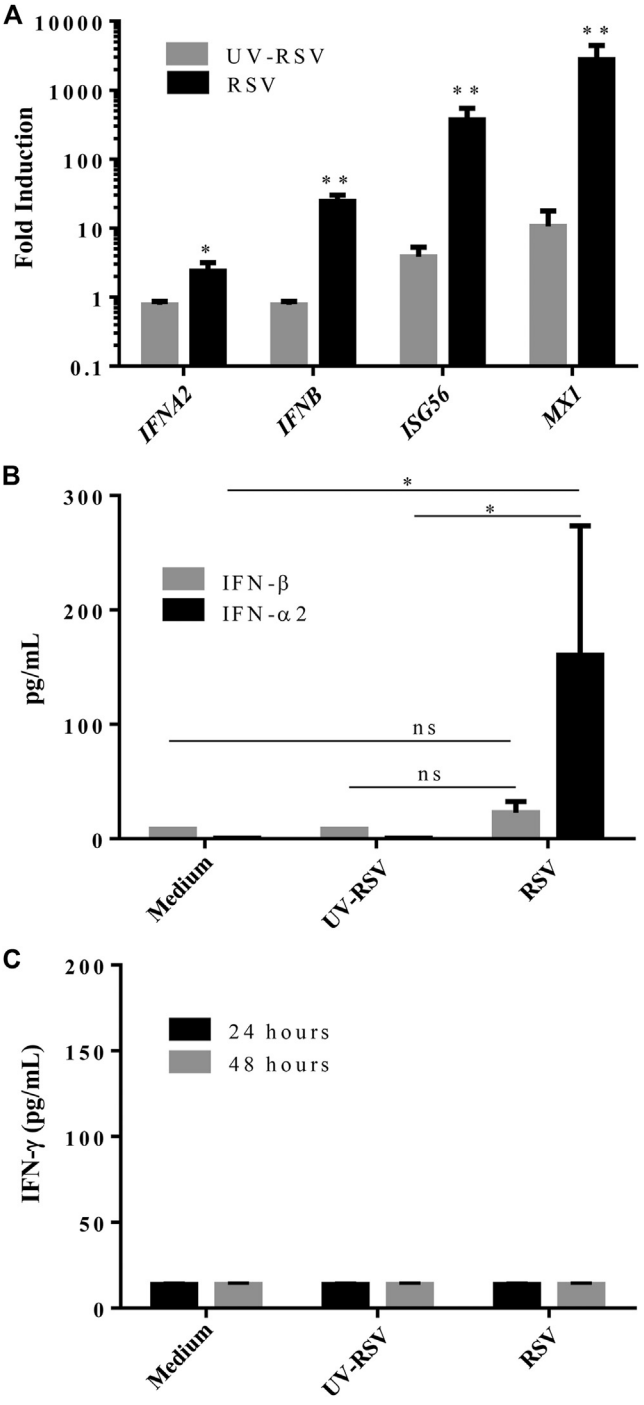

FIG 4. Type I and II interferon responses in CBMCs after infection with RSV. **A,** Fold induction of some type I interferons and interferon-stimulated genes at 24 hours in UV-RSV– and RSV-treated CBMCs relative to values seen in medium-treated CBMCs (n = 6 donors, mean ± SEM). **B,** IFN-α2 and IFN-β secreted from CBMCs 24 hours after infection. **C,** IFN-γ at 24 and 48 hours after CBMC infection with RSV (n = 5 donors, mean ± SEM in Fig 4, *B* and *C*). *$P < .05$ and **$P < .01$. *ns,* Not significant.

during RSV infection selectively inhibits production of CXCL10 and CCL4 but not CCL5 (Fig 5). These results are consistent with a model in which RSV infection of a subset of mast cells or an early mast cell response to signals associated with viral protein

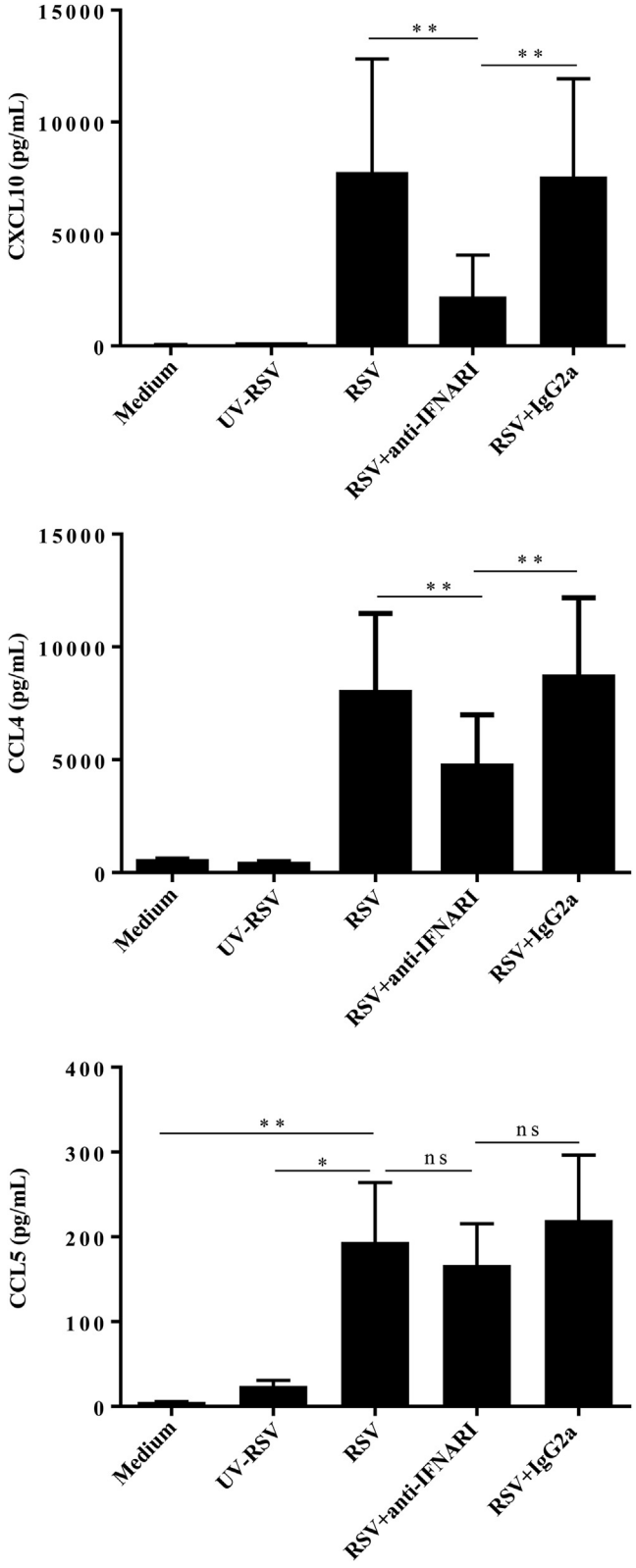

FIG 5. Effect of blocking type I interferon receptor on chemokine production by CBMCs during RSV infection at 24 hours (n = 7 donors, mean ± SEM). *$P < .05$ and **$P < .01$. *ns*, Not significant.

expression leads to the production of type I interferons that would then act on neighboring cells to induce selective CXCL10 and CCL4 production. Therefore, mast cells would contribute to an early antiviral state in surrounding cells and to the recruitment of antiviral effector cells, such as T cells, through both type I interferon and chemokine production. These activities could be further enhanced by interferons and other cytokine signals from the neighboring epithelium.[53] Our data suggest that the time course of chemokine production from mast cells after RSV exposure might be limited. By the 48-hour time point, there was a reduced response to RSV, which might reflect the limited exposure of cells to both new virus particles and mast cell–derived type I interferons. These results are in keeping with our observations that no progeny virus is observed from mast cells. It is possible that degradation of chemokines from mast cells by mast cell proteases might contribute to reduced chemokine detection at later time points. Because RSV is recognized to be a labile virus in cell culture and cells are washed after viral adsorption, there is little free virus available from the initial inoculum to provide a source for ongoing mast cell infection. This was confirmed by our plaque assay studies in which no infectious virus was observed from RSV-treated mast cell cultures.

Notably, the profile of chemokines produced by mast cells exposed to RSV overlaps considerably with that reported from virally infected pDCs, which have the capacity to induce the recruitment of T and NK cells.[54] Because mast cells, unlike pDCs, are resident in the human airways in substantial numbers, they might be particularly important for the initial chemokine response to infection. In addition, activated mast cells have also been shown to contribute to DC recruitment to inflammatory sites[55-58] and therefore could contribute to a coordinated DC and mast cell response to infection. CXCL10 has the ability to recruit macrophages and DCs and augment $CD8^+$ T-cell efficacy *in vivo* in mice and enhance type I interferon production from DCs *in vitro*.[59] CCL4 is also important in T-cell recruitment to inflamed sites and regulates the migration of monocytes, DCs, and NK cells.[60,61] CCL4 and CCL5 induce IL-12 production from DCs and have been shown to enhance a T_H1 response in a parasite model.[62]

The ability of RSV to infect human mast cells has previously been examined by using the HMC-1 cell line. This study concluded that mast cells could not be infected by RSV and only responded to RSV indirectly when cocultured with infected airway epithelial cells.[63] Our data clearly document viral gene expression and RSV protein expression in both CBMC and the HMC-1 cell line (Fig 1). The large amount of cellular activity devoted to cell division and growth, as well as the range of stages of cell cycle that are found within the HMC-1 cells might contribute to their reduced chemokine response, compared with CBMC. Their relatively immature mast cell phenotype may also limit their chemokine responses, directly or indirectly. HMC-1 cells are certainly not a perfect model for human *in vivo* mast cells. However, the HMC-1 data we provide does demonstrate that even 100% pure mast cells show a similar level of infection and range of chemokine responses as CBMC following RSV treatment. In keeping with our findings, RSV treatment of peripheral blood derived mast cells has also previously been reported to induce a type I IFN response.[64] Taken together these findings suggest that multiple sources of human mast cells might share the ability to enhance local antiviral immunity in response to RSV.

That we could not detect mature RSV progeny production in infected CBMC, could be due to the low levels of infection or due to nonproductive infection of mast cells as documented in influenza infection of mast cells.[65] In either case the primary

physiologic role of mast cells in patients with RSV infection is likely to enhance the recruitment of effector cells through mediator production, including type I interferons, CXCL10, and CCL4. The lack of any evidence of virus-induced LTC_4 production suggests little role for mast cells as a direct source of this mediator inducing bronchoconstriction; however, additional inflammatory factors could help promote mast cell activation and cysteinyl leukotriene production *in vivo*. Notably, in severe bovine RSV infection of calves considerable mast cell degranulation has been noted.[66] It is not clear if this is a direct consequence of RSV infection or a result of other aspects of the inflammatory pathology in this disease. In a separate study by Kimman et al,[67] mast cell degranulation was also observed in infected calves. However, complement activation and antiviral antibodies were also consistently observed. Therefore, both direct antibody mediated mechanisms and complement mediated pathways could contribute to the observed changes in mast cell granulation status. These studies of later stage disease suggest that the role of the mast cell might not be limited to the very early response to RSV infection, in the absence of specific antibody that we have examined experimentally.

Overall, our study suggests that, despite evidence of only limited RSV infection, human mast cells can support viral gene expression and have a role in RSV disease pathology and immunity through type I interferon and chemokine production. The ability of mast cells to produce significant levels of CCL4, CCL5, and CXCL10, which are associated with T-cell, NKT cell, and monocyte recruitment, but not CCL11 or CXCL8, which have major roles in eosinophil and neutrophil recruitment, suggests a role for mast cells in RSV control rather than damaging bronchiolitis. The long-lived resident nature of mast cells in the respiratory tract enables them to respond as sentinel cells very early to RSV infection and orchestrate the ensuing immune response. Through manipulation of human mast cell response to viruses, such as RSV, and to type I interferons, there might be opportunities to locally enhance effective early antiviral immunity and limit damaging inflammation associated with RSV infection.

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Key Messages

- **Despite limited RSV infection of human mast cells, RSV treatment results in substantial and selective type I interferon and chemokine responses (CXCL10, CCL4, and CCL5).**
- **RSV induction of CXCL10 and CCL4, but not CCL5, in human mast cells is dependent on the autocrine or paracrine action of type I interferons.**

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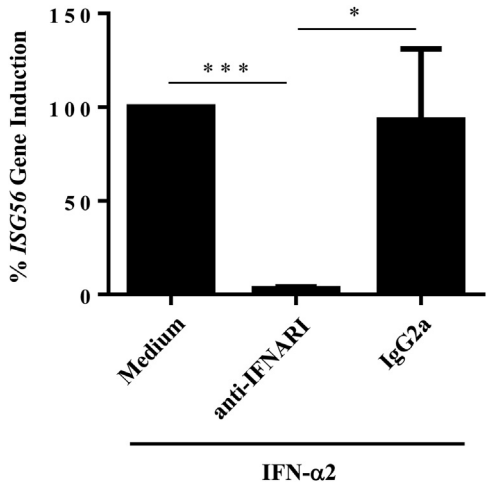

FIG E1. Induction of the interferon-stimulated gene *ISG56* by IFN-α2 at 24 hours with and without antibody blockade of the type I interferon receptor in KU812 cells to confirm functional efficiency of receptor blockade (n = 4, mean ± SEM). **P* < .05 and ****P* < .001.