GM-CSF treatment prevents respiratory syncytial virus–induced pulmonary exacerbation responses in postallergic mice by stimulating alveolar macrophage maturation

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Background: Human respiratory syncytial virus (RSV) is a frequent cause of asthma exacerbations, yet the susceptibility of asthmatic patients to RSV is poorly understood.
Objective: We sought to address the contribution of resident alveolar macrophages (rAMs) to susceptibility to RSV infection in mice that recovered from allergic airway eosinophilia.
Methods: Mice were infected with RSV virus after clearance of allergic airway inflammation (AAI). The contribution of post-AAI rAMs was studied *in vivo* by means of clodronate liposome–mediated depletion, adoptive transfer, and treatment with recombinant cytokines before RSV infection.
Results: After clearing the allergic bronchial inflammation, post-AAI mice had bronchial hyperreactivity and increased inflammatory cell influx when infected with RSV compared with nonallergic mice, whereas viral clearance was comparable in both mouse groups. Post-AAI rAMs were necessary and sufficient for mediating these proinflammatory effects. In post-AAI mice the residing CD11c^{hi} autofluorescent rAM population did not upregulate the terminal differentiation marker sialic acid–binding immunoglobulin-like lectin F and overproduced TNF and IL-6 through increased nuclear factor κB nuclear translocation. In line with these results, post-AAI lungs had reduced levels of the rAM maturation cytokine GM-CSF. Intratracheal administration of GM-CSF induced final rAM maturation in post-AAI mice and prevented the increased susceptibility to RSV-induced hyperreactivity and inflammation.
Conclusion: Defective production of GM-CSF leads to insufficient post-AAI rAM maturation in mice that recovered from an AAI, causing increased susceptibility to RSV-induced immunopathology. Promoting the differentiation of post-AAI rAMs might be a therapeutic option for preventing RSV-induced exacerbations in human asthmatic patients. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Allergic asthma, respiratory syncytial virus–induced exacerbation, alveolar macrophage, GM-CSF*

Allergic asthma is a chronic inflammatory disease of the airways affecting more than 300 million persons worldwide. In asthmatic patients pulmonary accumulation of inflammatory leukocytes and cytokines together with lung structural remodeling and airway hyperreactivity (AHR) eventually results in recurrent episodes of airway obstruction, wheezing, and shortness of breath, which are clinically referred to as exacerbation reactions.[1] Although the mechanisms of asthmatic exacerbations are still poorly understood, it is increasingly clear that the majority of exacerbations are associated with acute viral respiratory tract infections, including rhinoviruses and influenza. However, the most severe asthmatic exacerbation reactions are often induced by respiratory syncytial virus (RSV) infections.[2]

RSV is the leading cause of infant hospital admission worldwide and causes 70% of bronchiolitis-associated hospitalizations in the developed world.[3] Although CD8$^+$ T cells[4,5] and neutralizing antibodies[5-7] are pivotal for viral clearance, a number of studies emphasize the importance of alveolar macrophages as key players in the early immune response to RSV infection.[8,9] It has even been suggested that alveolar macrophages, rather than adaptive immune cells, are critical determinants of the severity of RSV-induced bronchiolitis.[9]

Resident alveolar macrophages (rAMs) constitute the predominant cell population in the alveolar spaces of healthy subjects and serve as important innate sentinels in the recognition of invading pathogens and other airborne particles. However, in rAMs these innate responses are tightly regulated, thus avoiding excessive inflammatory responses that can hamper pulmonary gas exchange.[10] However, inflammatory insults as such can also negatively affect the anti-inflammatory function of rAMs. In the case of allergic inflammation, we recently reported the presence of a hyperinflammatory rAM phenotype exhibiting increased proinflammatory reactivity on exposure to Toll-like receptor (TLR) 3, TLR4 and TLR7 ligands.[11]

We have now verified to what extent the increased inflammatory reactivity of rAMs instigated by a prior allergic bronchial inflammation might affect the outcome of a subsequent infection

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Abbreviations used
AAI: Allergic airway inflammation
AHR: Airway hyperreactivity
BAL: Bronchoalveolar lavage
DC: Dendritic cell
DMEM: Dulbecco modified eagle medium
HBSS: Hank balanced salt solution
IRF: Interferon regulatory factor
NF-κB: Nuclear factor κB
OVA: Ovalbumin
PE: Phycoerythrin
PFA: Paraformaldehyde
PFU: Plaque-forming units
rAM: Resident alveolar macrophage
RSV: Respiratory syncytial virus
RT: Room temperature
Siglec-F: Sialic acid–binding immunoglobulin-like lectin F
TLR: Toll-like receptor

with RSV. Our results show that post–allergic airway inflammation (post-AAI) lungs are prone to increased pulmonary inflammation and AHR in response to RSV infection and that the hyperinflammatory phenotype of post-AAI rAMs is at the origin of the observed inflammation exacerbation in post-AAI mice. Furthermore, we found that the majority of the post-AAI rAM population displayed an immature phenotype. Full maturation of this immature post-AAI rAM population by GM-CSF treatment prevented the exacerbated nature of the lung response to RSV infection, resulting in decreased bronchoalveolar inflammation and AHR after RSV infection.

METHODS

Mice

Six- to 8-week old female BALB/c mice were purchased from Charles River Laboratories Italy (Calco, Italy) and housed under specific pathogen-free conditions in individually ventilated cages in a controlled day/night cycle and given food and water *ad libitum*. All experiments were approved by the local animal ethics committee of Ghent University in accordance with European guidelines (directive 2010/63/EU; Belgian Royal Decree of April 6, 2010).

Mouse model of RSV-induced inflammation exacerbation in allergen-sensitized and challenged mice

A mouse model of RSV-induced inflammation exacerbation in allergen-sensitized and challenged mice was established, as described in the Methods section in this article's Online Repository at www.jacionline.org. In some experiments mice were treated with recombinant GM-CSF (provided by the VIB Protein Service Facility, Ghent, Belgium; see the Methods section in this article's Online Repository).[12]

Alveolar cell isolation, cell culture, and differential cell counts

Bronchoalveolar lavage (BAL) and *ex vivo* cultures of rAMs incubated for the indicated time with RSV A2 were performed, as described in the Methods section in this article's Online Repository. Total and differential numbers of BAL cells were determined by means of flow cytometry, as described in the Methods section in this article's Online Repository.

Determination of AHR

Lung function was assessed by using flexiVent (SCIREQ, Montreal, Quebec, Canada) invasive measurement of dynamic lung compliance, as described in the Methods section in this article's Online Repository.

rAM depletion and reconstitution

Depletion of the residing alveolar phagocyte population was achieved by means of intratracheal instillation of a 30% solution of clodronate-filled liposomes (N. Van Rooijen, VUC Amsterdam, Amsterdam, The Netherlands), as described in the Methods section in this article's Online Repository.

Cytokine measurement

Protein levels of mouse IL-6 and TNF in BAL fluid and culture supernatants were quantified with the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, Calif).

Total RNA preparation and real-time quantitative PCR

RNA isolation was performed with the RNeasy Plus Micro Kit (Qiagen, Hilden, Germany). cDNA was synthesized with an iScript cDNA synthesis kit (Bio-Rad Laboratories). Real-time quantitative PCR was performed on a LightCycler 480 (Roche Molecular Systems, Basel, Switzerland) by using the sensiFAST SYBR No-ROX kit (Bioline, London, United Kingdom). Primers for ubiquitin-specific peptidase 18 *(Usp18)*, interferon-induced protein with tetratricopeptide repeats 2 *(Ifit2)*, and 60S ribosomal protein L13 a (*Rpl13a*; reference housekeeping gene for normalization) were used.

Nuclear translocation assays

BAL cells were infected *in vitro* with RSV. At the indicated time points, cells were stained intracellularly for nuclear factor κB (NF-κB) p65, interferon regulatory factor (IRF) 3, or IRF-7, as described in the Methods section in this article's Online Repository. Stained cells were imaged with a Leica TCS SP5 AOBS confocal microscope (Leica, Wetzlar, Germany). Images were acquired with LAS AF software (Leica) and subsequently analyzed with Volocity software (PerkinElmer, Beaconsfield, United Kingdom).

Statistics

Statistical analysis was performed with GraphPad Prism 5 software (GraphPad Software, La Jolla, Calif). Following outlier statistics, the Kolmogorov-Smirnov test was used to check Gaussian distribution of parameters to choose between 1-way ANOVA and the Kruskal-Wallis nonparametric test. Differences in means between each 2 independent experimental groups were analyzed by using an unpaired t test or the nonparametric Mann-Whitney U test at a 95% CI. No statistical analysis was done for data of pooled samples.

RESULTS

Post-AAI mice have increased pulmonary inflammation in response to RSV infection

A mouse model of AAI was set up to examine the influence of a preceding allergic bronchial inflammation on a subsequent pulmonary RSV infection. BALB/c mice were sensitized against the model allergen ovalbumin (OVA) by means of repeated intraperitoneal immunization with aluminum hydroxide as an adjuvant. Subsequently, the sensitized mice were repeatedly exposed to nebulized OVA, generating an eosinophilic airway inflammation reminiscent of the immunopathology of mild-to-moderate asthma (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org).