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Double-Stranded RNA Exacerbates Pulmonary Allergic Reaction through TLR3: Implication of Airway Epithelium and Dendritic Cells

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Respiratory viral infections have been implicated in exacerbations of allergic asthma, characterized by a Th2-biased immune response. Respiratory viruses target airway epithelial cells and dendritic cells (DCs). Their activation is, at least in part, mediated by the TLR3-dependent recognition of virus-derived dsRNA. To elucidate the role of epithelial cells and DCs and the implication of TLR3/Toll/IL-1R domain-containing adaptor-inducing IFN- β (TRIF) pathway, we developed a mouse model of lung allergic exacerbation. The effect of intranasal administration of dsRNA in OVA-sensitized wild-type mice and TRIF^{-/-} mice was evaluated on airway hyperresponsiveness and pulmonary inflammation. Our data demonstrated that treatment with dsRNA significantly increased the airway hyperresponsiveness, the lung inflammation, and the OVA-specific Th2 response. This was associated with an infiltrate of eosinophils, myeloid DCs, and T lymphocytes. TRIF activation was required for the development of dsRNA-induced exacerbation of the allergic reaction. Intratracheal transfer of IL-4/dsRNA/OVA-pretreated DCs also triggered exacerbation of the allergic reaction, whereas cells primed with dsRNA/OVA had a more limited effect. dsRNA-induced production of CCL20 by airway epithelium was associated with DC recruitment. In vivo and in vitro treatment with dsRNA amplified airway epithelial production of the pro-Th2 chemokines CCL11 and CCL17, their secretion being enhanced by Th2 cytokines. In conclusion, dsRNA derived from respiratory viruses trigger exacerbation of the pulmonary allergic reaction through TLR3/TRIF-dependent pathway. Moreover, Th2 cytokines participate in this process by modulating the response of airway epithelium and DCs to dsRNA. *The Journal of Immunology*, 2010, 185: 000–000.

Allergic asthma is a complex inflammatory disease associated with mucus hypersecretion, remodeling, airway hyperresponsiveness (AHR), eosinophil, and Th2 cell infiltrates in the lung (1–4). Ag-induced allergic airway

inflammation is mediated by Th2 cytokines, such as IL-4, IL-5, and IL-13 (1–4). IL-4 is important for Th2 cells and IgE response development, whereas IL-13 induces airway remodeling and AHR as well as goblet cell hyperplasia (5, 6). IL-5 mainly mediates eosinophil recruitment into the airways of sensitized mice (7, 8). A critical role for dendritic cells (DCs) is now fairly established in allergen sensitization, airway inflammation, and AHR alteration associated with experimental models of allergic asthma (9, 10).

Airway epithelial cells (AECs) are involved in the control of lung inflammation and DC recruitment during allergic asthma (11). AECs exposed to airway contaminants (virus, bacteria, and pollutants) produce higher levels of chemokines (MIP3- α [CCL20], IP-10 [CXCL10], and RANTES [CCL5]) involved in the recruitment of myeloid (m)DCs (12, 13). Airway exposure to TLR agonists also affects the expression of adhesion molecules on AECs as well as the production of cytokines involved in their cross-talk with mDCs. This leads to the maturation of mDCs and the polarization of the T cell response toward a Th2 profile (12, 14). Furthermore, AECs drive allergic inflammation to house dust mites via TLR4-dependent activation of mucosal mDCs (15).

Viral airway infections by influenza A virus, rhinovirus, and respiratory syncytial virus are responsible for the majority of asthma exacerbation in children (16–18). In nonatopic mice, these infections induce a Th1 immune response (19). However, infections with respiratory viruses in allergic mice exacerbate Th2 pathologies (20–22). Influenza A virus infection promotes the maturation of lung DCs and leads to increased Th2 response (22).

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Abbreviations used in this paper: AEC, airway epithelial cell; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; BMDC, bone marrow-derived dendritic cell; DC, dendritic cell; m, myeloid; LN, lymph node; MHC II, MHC class II; PenH, enhanced pause; RL, airway resistance; TRIF, Toll/IL-1R domain-containing adaptor-inducing IFN- β ; WT, wild-type.

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Induction of immune responses by respiratory viruses involves pattern recognition receptors located either in endosomes, such as TLRs 3, 7, 8, and 9, or present in the cytoplasm including the RNA helicases RIG-I and MDA-5 (23, 24). Both TLR3 and RNA helicases recognize dsRNA produced during viral infections as well as poly(I:C), a synthetic ligand mimicking viral dsRNA (25–28). This recognition induces activation of NF- κ B and IFN regulatory factor-3/7 transcription factors that further regulate inflammatory and antiviral mediator expression. In virus-infected AECs, secretion of proinflammatory chemokines is dependent on TLR3 through the mobilization of the adaptor molecule Toll/IL-1R domain-containing adaptor-inducing IFN- β (TRIF) (29), whereas retinoic acid-inducible gene-I controls the production of type I IFN (30). In DCs, involvement of TLR3 versus RNA helicases depends on the targeted subpopulation and their degree of activation (31, 32).

Although several studies showed an exacerbation of allergic asthma after viral infections, the cellular and molecular processes involved in this disease are still unknown. For this purpose, we developed a mouse model for allergic reaction exacerbation based on the local administration of dsRNA. In this study, we investigated the potential role of AEC/DC interactions and the implication of TLR3/TRIF pathway in exacerbation of the allergic reaction. Our data show that the TLR3/TRIF pathway is involved in lung allergic exacerbation and suggests the importance of AEC/mDC cross-talk in this setting.

Materials and Methods

Animals

C57BL/6 mice were purchased from Charles River Laboratories (Le Genest-St-Isle, France). TRIF^{-/-} mice and TLR3^{-/-} mice (a gift from Prof. S. Akira, Osaka University, Osaka, Japan) were backcrossed on the C57BL/6 background (Unité Mixte de Recherche 6218, Orleans, France) and were bred in a specific pathogen-free animal facility at the Centre National de la Recherche Scientifique. For experiments, female (8–10 wk old) animals were kept isolated in ventilated cages. All protocols complied with the French government's ethical and animal experiment regulations.

Model of pulmonary allergic reaction exacerbation

Wild-type (WT) mice and TRIF^{-/-} mice were sensitized with 50 μ g OVA (Sigma-Aldrich, St. Louis, MO) in 2 mg aluminum hydroxide i.p. in a volume of 200 μ l on days 0 and 7 (Fig. 1A). From days 14 to 16, anesthetized (with xylazine/ketamine) mice were challenged intranasally with 100 μ g OVA (Sigma-Aldrich) diluted in PBS alone or in association with 50 μ g endotoxin-free poly(I:C) (dsRNA; InvivoGen, San Diego, CA) to induce allergic airway inflammation. dsRNA preparations are devoid of endotoxin as determined by the *Limulus* amoebocyte test and did not activate a cell line transfected with TLR4 (data not shown). Controls received PBS alone. Ten days after intranasal administration, mice were challenged with OVA aerosol (OVA 2% in PBS for 20 min) on 3 consecutive days. Animals were sacrificed 48 h after the last challenge. Lung, sera, and bronchoalveolar lavage (BAL) fluids were then collected for analysis. In some experiments, mice were sacrificed after the first set of challenge (at day 17) to analyze the lung recruitment of innate immune cells induced by dsRNA.

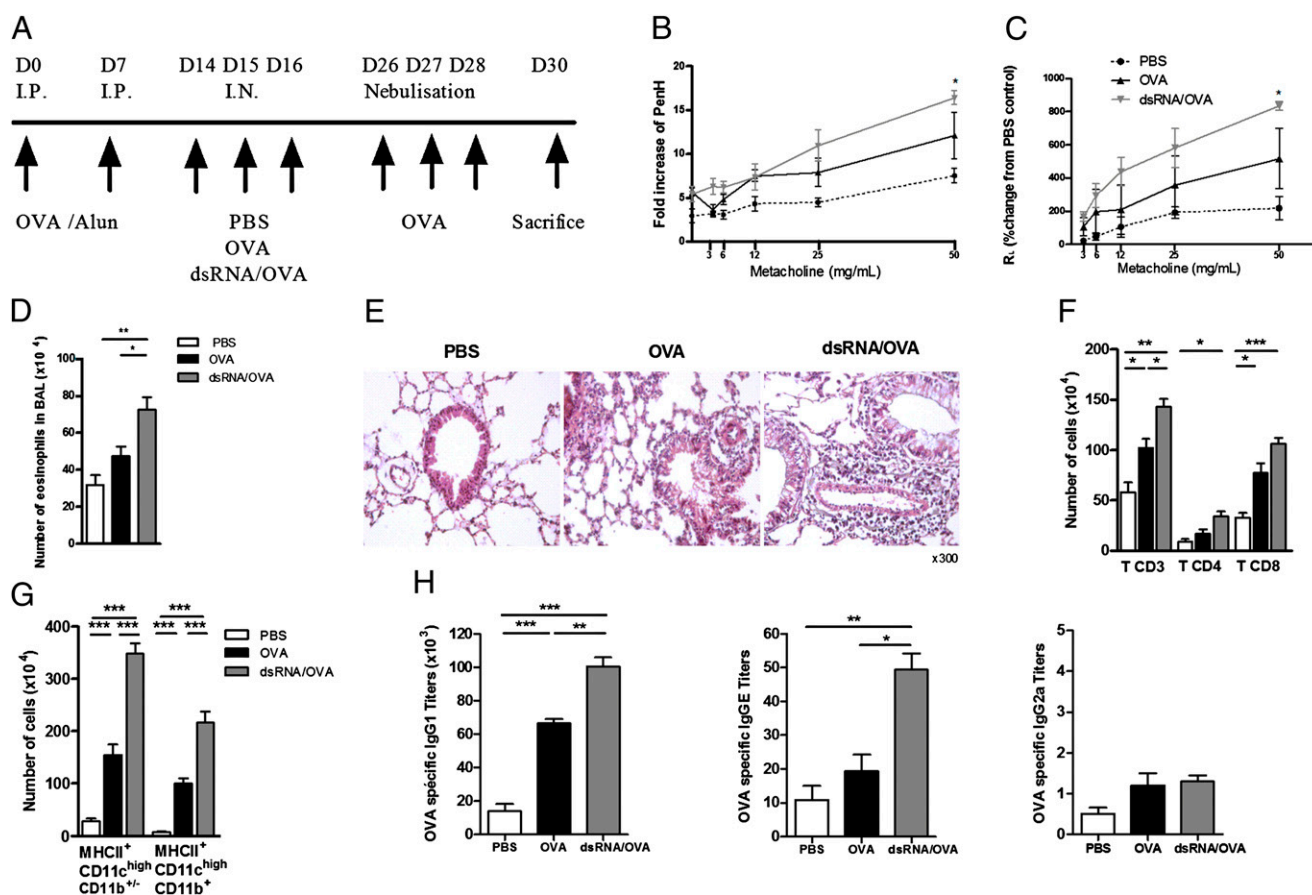


FIGURE 1. Local administration of dsRNA exacerbates the lung allergic reaction in WT C57BL/6 mice. **A**, Protocol for exacerbation of the allergic reaction induced by dsRNA administration in C57BL/6 mice. **B** and **C**, Lung function, as determined by measurement of PenH (**B**) and RL (**C**) by noninvasive and invasive methods, respectively (* p < 0.05 versus mice treated with OVA); **D**, eosinophil numbers in the BAL; **E**, H&E staining of lung sections (original magnification $\times 300$); **F** and **G**, number of T cells (**F**) and DCs (**G**) in lung tissue as determined by flow cytometry. In **G**, DCs are defined as nonautofluorescent MHCII⁺CD11c^{high}CD11b^{-/-} and MHCII⁺CD11c^{high}CD11b⁺, respectively; **H**, serum OVA-specific IgG1, IgE, and IgG2a levels. Data are expressed as the mean $\pm$ SD (n = 5 mice) and are from one representative experiment out of three. * p < 0.05; ** p < 0.01; *** p < 0.001 versus mice treated either with OVA or PBS.

Measurement of AHR

Measurement of AHR was first performed by a noninvasive method using whole-body plethysmography and then confirmed by an invasive method using flexivent (Scireq, Montreal, Quebec, Canada). Responsiveness to β -metacholine was assessed in conscious mice using the measurement of enhanced pause (PenH) by chamber whole-body plethysmography. Alternatively, airway resistance (RL) and dynamic compliance was measured by flexivent.

Generation of mDCs and adoptive transfer of OVA-pulsed DCs

In brief, bone marrow-derived DCs (BMDCs) were differentiated in the presence of GM-CSF (20 ng/ml; PeproTech, London, U.K.) (see supplemental *Extended Methods* section for further details). After 10 d, a pro-Th2 environment was constituted by addition of IL-4 (10 ng/ml) (PeproTech) into some cultures. At day 13, both types of BMDCs were stimulated with OVA (100 ng/ml) alone or in association with dsRNA (10 μ g/ml). Control cells were incubated in medium alone. After 24 h of incubation, BMDC phenotype was analyzed by flow cytometry and for its capacity to produce cytokines. To address the role of DCs in asthma exacerbation, C57BL/6 mice were sensitized as described above. At day 14, 10^6 BMDCs (sensitized or not with IL-4) were injected intratracheally and then challenged with OVA aerosol (days 26–28) before evaluation of the allergic reaction.

Assessment of allergic airway inflammation in BALs, lung tissue, and draining lymph nodes

BAL fluids were prepared by washing the lungs twice with 0.5 ml ice-cold PBS. Cell-free BAL fluids and the cell pellets were prepared and used for differential cell counts and measurement of cytokine levels. Lung homogenates were prepared after dispersion of the middle right lobe in 1 ml lysis buffer (see *Extended Methods* for further details). The concentration of the total protein and cytokine levels was measured using protein assay (Bio-Rad, Hercules, CA) and ELISA (R&D Systems, Abingdon, U.K.), respectively. After dissociation of the left lobe, lung cells were counted, and the cell phenotype was analyzed by flow cytometry. The gating strategy to identify DCs was described in *Extended Methods* and in Supplemental Fig. 1. For lung histology and immunohistochemistry, caudal right lobe of the lung from each mouse was fixed in Immunohistofix and embedded in resin using the Immunohisto wax processing method (A PHASE, Gosselies, Belgium). Inflammatory infiltrate was analyzed on lung sections stained with H&E. CCL11, CCL17, and CCL20, as well as MHC class II (MHC II)-positive cells, were also studied by immunohistochemistry on lung sections.

Mediastinal lymph node (LN) cells (3×10^5 /well) were plated and restimulated with 20 μ g/ml OVA and 5 μ g/ml fixed anti-mouse CD3 mAb (BD Biosciences, San Jose, CA). Cell supernatants were collected 4 d later. In some cases, mediastinal LN cells were analyzed by flow cytometry.

Determination of OVA-specific serum Ab concentration

OVA-specific IgG1, IgG2a, and IgE serum levels were measured by ELISA as previously described (33) with some modifications (see *Extended Methods* for further details). Results were expressed as the inverse of the dilution corresponding to 50% of the maximal OD.

Murine AECs

After sacrifice of WT mice and TLR3^{-/-} mice, AECs were prepared by enzymatic digestion within the airways (see *Extended Methods* for further details). After cell culture, >95% of AECs stained for cytokeratin and were negative for vimentin (data not shown) (Sigma-Aldrich). After cell starvation, AECs were activated by dsRNA (10 μ g/ml) for 24 h before cell supernatant collection. In some cases, AECs were presensitized with recombinant mouse IFN- γ (10 ng/ml) or IL-4 plus IL-13 (10 ng/ml) (PeproTech) for 18 h before activation.

Measurement of cytokine levels

Levels of IL-4, IL-5, IFN- γ , IL-13, CCL11, CCL17, CCL20, CXCL10, and CCL5 were measured by ELISA (R&D Systems) in BAL fluids and lung extracts and in the supernatants of AECs, BMDCs, and mediastinal LN cells.

Chemotaxis assays

Chemotaxis assays were performed using 24-well Transwells (5- μ m-pore polycarbonate filter) (Corning, Avon, France) (see *Extended Methods* for further details). After 2 h at 37°C, the number of migrated BMDCs (CD11c⁺MHC II⁺) was numerated and identified by flow cytometry. The

implication of CXCL10, CCL5, CCL11, and CCL20 in the chemotactic activity of AEC supernatant was determined by using specific neutralizing Abs (R&D Systems).

Statistical analysis

Results are expressed as the mean $\pm$ SD. Statistical significance of the differences between experimental groups was calculated by ANOVA with a Bonferroni posttest or an unpaired Student *t* test (GraphPad Prism 4 Software; GraphPad, San Diego, CA). Results with a value of *p* < 0.05 were considered as significant. The in vivo experiments were performed two or three times with at least five mice per group. One representative experiment out of these replicates is shown.

Results

Administration of dsRNA in OVA-sensitized mice induces exacerbation of the pulmonary allergic reaction

Allergic asthma is a complex, multifactorial inflammatory disease that may be exacerbated by viral infection. To reproduce virus-induced exacerbation of asthma, OVA-sensitized mice were challenged intranasally with OVA in the presence of dsRNA, a viral mimetic, at days 14–16 and then rechallenged with OVA alone at days 26–28 (Fig. 1A). As seen in Fig. 1, an asthma-like phenotype with AHR and eosinophilic airway inflammation was induced after intrapulmonary challenge with OVA alone. Interestingly, relative to the mice challenged with OVA alone, dsRNA/OVA administration induced a significant increase of AHR as measured by PenH in a whole-body plethysmograph (Fig. 1B) and by direct measurement of RL and dynamic compliance in anesthetized, tracheotomized, intubated, and mechanically ventilated mice (Fig. 1C). Treatment with dsRNA/OVA was also associated with more total cells,

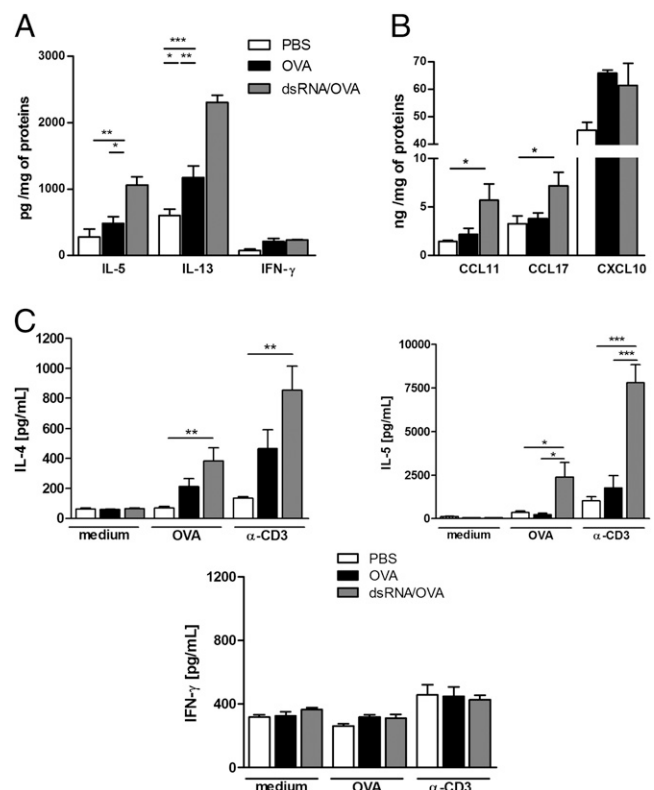


FIGURE 2. Local administration of ds-RNA increases Th2 responses in allergic C57BL/6 mice. *A* and *B*, Levels of cytokines (*A*) and chemokines (*B*) in protein extract of lung tissue as determined by ELISA; *C*, cytokine concentrations in supernatants from draining LN cells restimulated with OVA or anti-CD3 mAb. Data are expressed as the mean $\pm$ SD (*n* = 5 mice) and are from one representative experiment out of three. **p* < 0.05; ***p* < 0.01; ****p* < 0.001 versus mice treated either with OVA or PBS.

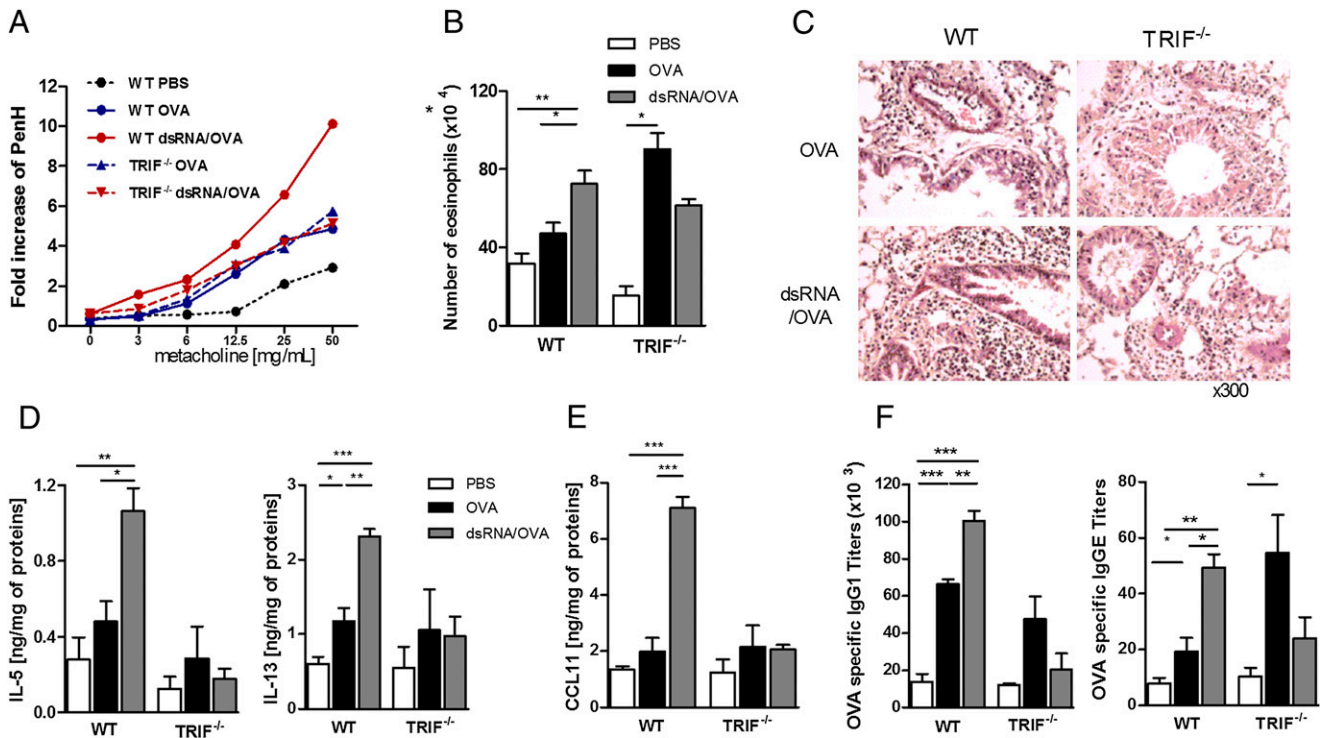


FIGURE 3. TRIF expression is necessary for dsRNA-induced exacerbation of the lung allergic reaction. **A**, Lung function, determined by measurement of PenH; **B**, absolute number of eosinophils in the BAL; **C**, H&E staining of lung sections (original magnification $\times 300$); **D** and **E**, cytokine (**D**) and chemokine (**E**) levels in lung extracts as determined by ELISA. **F**, Serum OVA-specific IgG1 and IgE levels. Data are expressed as the mean $\pm$ SD ($n = 5$ mice) and are from one representative experiment out of two. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus mice treated either with OVA or PBS.

including eosinophils, in the BAL fluids (Fig. 1D and data not shown). Moreover, dsRNA/OVA treatment enhanced the infiltration of peribronchial cell in the BAL fluids, which are mostly composed of eosinophils and mononuclear cells (Fig. 1E). In parallel, as revealed by flow cytometry, a strong infiltration of CD4⁺ and CD8⁺ lymphocytes, as well as lung DCs (MHC II⁺CD11c⁺CD11b^{+/−}), was observed in the lungs (Fig. 1F and 1G) and in the mediastinal LNs (data not shown) of dsRNA-treated mice, relative to animals challenged with OVA alone. Among DCs, ~70% expressed the CD11b molecule. Interestingly, it appears that the dsRNA treatment also heightened serum titers of OVA-specific IgG1 and IgE but not IgG2a (Fig. 1H).

To further investigate the impact of dsRNA treatment, we monitored the nature of the local immune response. Analysis of Th2-type cytokine concentrations in lung tissues revealed that,

relative to mice challenged with OVA alone, a higher level of IL-5 and IL-13 was found in mice treated with OVA plus dsRNA (Fig. 2A). In contrast, no significant difference in IFN- γ was observed between the two groups. Furthermore, chemokine levels associated with Th2 response, such as CCL11 and CCL17, were more abundant in mice receiving dsRNA/OVA in comparison with mice receiving OVA alone (Fig. 2B). In contrast, dsRNA administration did not modulate the level of the Th1-related chemokine CXCL10. The polarization of the T cell response was then analyzed, and mediastinal LN cells were restimulated with OVA or anti-CD3, and cytokine production was assessed. As depicted in Fig. 2C, higher levels of IL-4 and IL-5 were found in the supernatants of cells prepared from dsRNA/OVA mice, as compared with cells obtained from mice only challenged with OVA. The levels of IFN- γ were not changed.

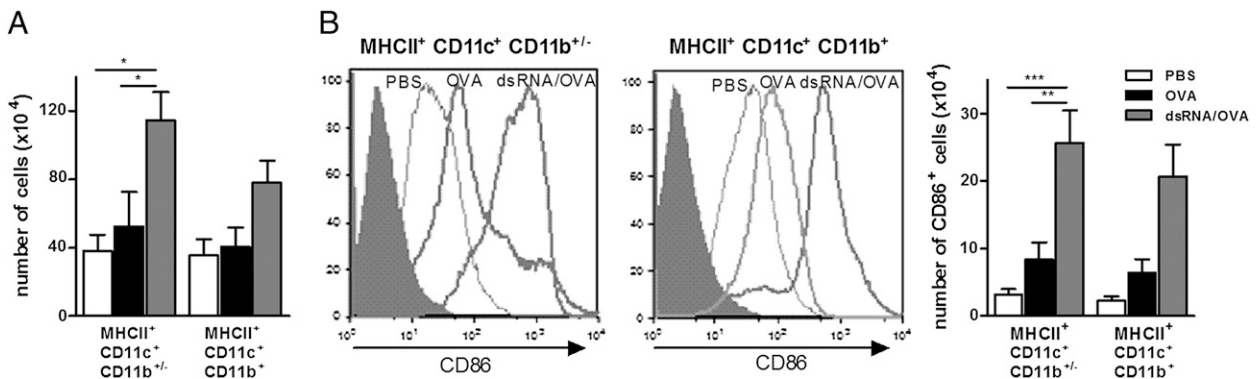


FIGURE 4. Local administration of dsRNA induces recruitment and maturation of mDCs. **A**, Treatment with dsRNA/OVA (from days 14 to 16) increased at day 17 the number of DCs (MHC II⁺CD11c⁺CD11b^{+/−} and MHC II⁺CD11c⁺CD11b⁺) within the lung of sensitized mice; **B**, treatment with dsRNA/OVA enhanced at day 17 the level of CD86 expression and the number of CD86⁺ DCs within the lung of sensitized mice. Data are expressed as one representative experiment out of three and as the mean $\pm$ SD ($n = 5$ mice). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus the indicated condition.

Overall, local administration of dsRNA during the primary challenge phase induces exacerbation of the pulmonary allergic reaction, a phenomenon associated with an enhanced Th2 response in the lungs.

TRIF mobilization is involved in the dsRNA-induced exacerbation of the allergic reaction

Activation of TLR3, through TRIF signaling, plays an essential role in the inflammatory response induced by dsRNA exposure (34, 35). To determine the role of this signaling pathway in our experimental model, OVA-sensitized WT mice and TRIF^{-/-} mice were challenged with or without dsRNA. Although OVA exposure in TRIF^{-/-} mice induced allergic reaction, dsRNA/OVA administration did not exacerbate the reaction as seen in WT animals. As depicted in Fig 3A, and relative to WT animals, treatment with dsRNA did not enhance AHR in TRIF^{-/-} mice. The numbers of eosinophils in the BAL (Fig. 3B) as well as the levels of the peribronchial and perivascular cellular infiltrate (Fig. 3C) were not increased in TRIF^{-/-} mice. Of note, dsRNA did not increase the recruitment of T lymphocytes and lung DCs in the lungs of TRIF^{-/-} mice (data not shown). In TRIF^{-/-} mice compared with WT mice, dsRNA/OVA administration did not amplify the synthesis of IL-5, IL-13 (Fig. 3D), CCL11, CCL17 (Fig. 3E and data not shown), the titer of OVA-specific IgG1 and IgE (Fig. 3F), or the level of IgG2a (data not shown). These results indicate that TRIF signaling is essential in the dsRNA-induced exacerbation of the pulmonary allergic reaction.

Transfer of dsRNA-stimulated IL-4/BMDCs exacerbates the pulmonary allergic reaction

Because mDCs play a critical role in experimental models of pulmonary allergic reaction (10, 36), we evaluated the potential ability of dsRNA-treated DCs to mediate asthma exacerbation. We first analyzed the recruitment of DCs into the lungs immediately after dsRNA/OVA administration at day 17. As observed after the secondary OVA challenge (Fig. 1G), dsRNA/OVA administration during the primary challenge strongly promoted the recruitment toward the lung of MHC II⁺CD11c⁺CD11b⁺ cells (Fig. 4A). Moreover, the expression level of the costimulatory molecule CD86 was strongly enhanced on both CD11b⁺ and CD11b⁺ lung DCs from mice that received dsRNA (Fig. 4B).

To study the involvement of mDCs in our experimental model, in vivo transfer experiments were performed. The mDC was differentiated in vitro from bone marrow progenitors in the presence (IL-4/BMDCs) or absence (BMDCs) of IL-4. As seen in Fig. 5A and 5B, exposure of IL-4/BMDCs with dsRNA/OVA resulted in an enhanced synthesis of CD80 and CD86. This, however, was not the case for cells differentiated without IL-4. For both BMDC preparations, dsRNA/OVA exposure did not induce the production of the Th1-promoting cytokine IL-12p70. The synthesis of CCL5 was increased by dsRNA/OVA addition in both preparations (Fig. 5C).

We next evaluated the impact of DC transfer (day 14) on the pathology. The transfer of OVA-pulsed BMDCs did not modulate AHR, whereas the mice receiving dsRNA-stimulated IL-4/BMDCs, and to a lesser extent BMDCs, developed a significantly higher AHR (Fig. 6A). In parallel, dsRNA/OVA-primed IL-4/BMDCs promoted strong recruitment of eosinophils and mononuclear cells (including CD4⁺ and CD8⁺ T lymphocytes) in the lungs and in the BAL, an effect that was far more potent compared with BMDCs (Fig. 6B, 6C, Supplemental Table I). Higher levels of IL-5 and IL-13 but not IFN- γ (Fig. 6D) were found in the lungs of mice that received dsRNA/OVA-pulsed IL-4 BMDCs, compared with the animals receiving OVA-pulsed IL-4 BMDCs.

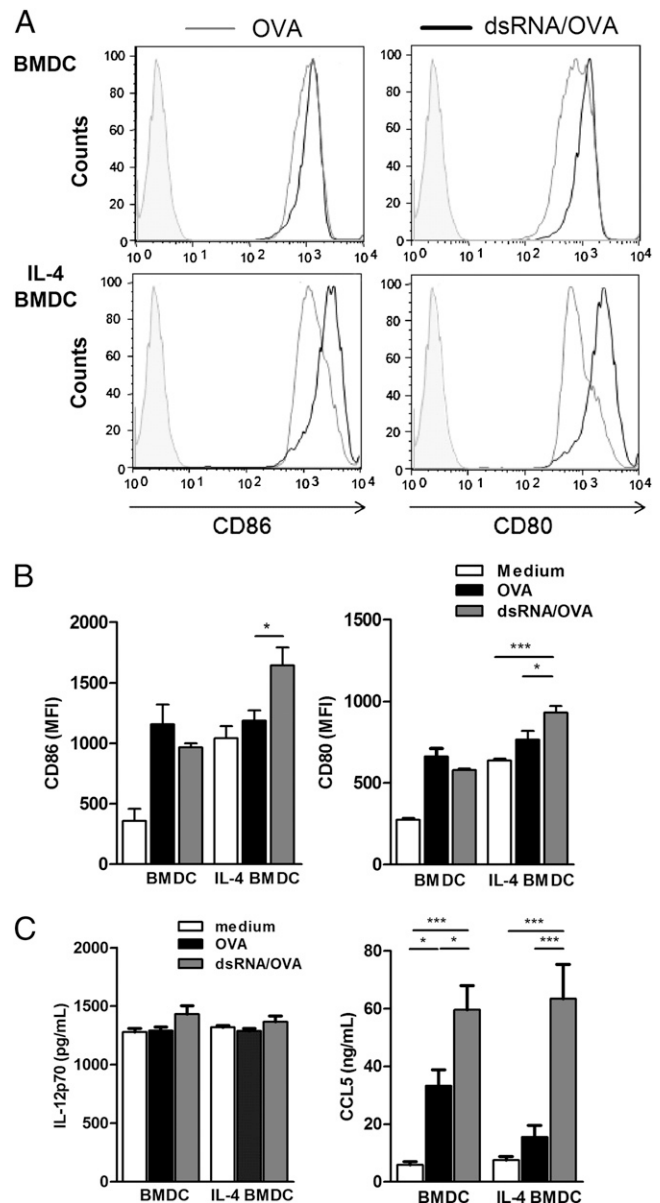


FIGURE 5. BMDC differentiation with Th2 cytokines increases the dsRNA-induced expression of costimulatory molecules. *A*, Representative experiment evaluating the expression of CD86 and CD80 by flow cytometry in BMDCs differentiated with GM-CSF (BMDCs) or with GM-CSF plus IL-4 (IL-4 BMDCs); *B*, the mean $\pm$ SD of CD86 and CD80 expression in BMDCs and IL-4 BMDCs is also reported; *C*, differentiation with IL-4 does not modulate the IL-12p70 and CCL5 secretion by dsRNA-activated BMDCs and IL-4 BMDCs ($n = 4$). Data are given as the mean $\pm$ SD of four independent experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus the indicated condition.

CCL11 and CCL17 levels were also significantly increased in mice that received dsRNA/OVA-pulsed IL-4 BMDCs (Fig. 6E). In contrast, dsRNA/OVA-stimulated BMDC transfer had no significant effect on cytokine production. Exposure of IL-4 BMDCs with dsRNA also enhanced the production of OVA-specific IgG1 and IgE but did not affect IgG2a synthesis (Fig. 6F).

We next analyzed cytokine secretion by mediastinal LN cells after restimulation with OVA or anti-CD3. When we compared mice treated with BMDCs and IL-4/BMDCs plus OVA with mice transferred with nonpulsed BMDCs, cell restimulation with OVA and anti-CD3 slightly increased IL-4 and IL-5 but not IFN- γ levels (Supplemental Fig. 2). For restimulated LN cells, BMDC treatment

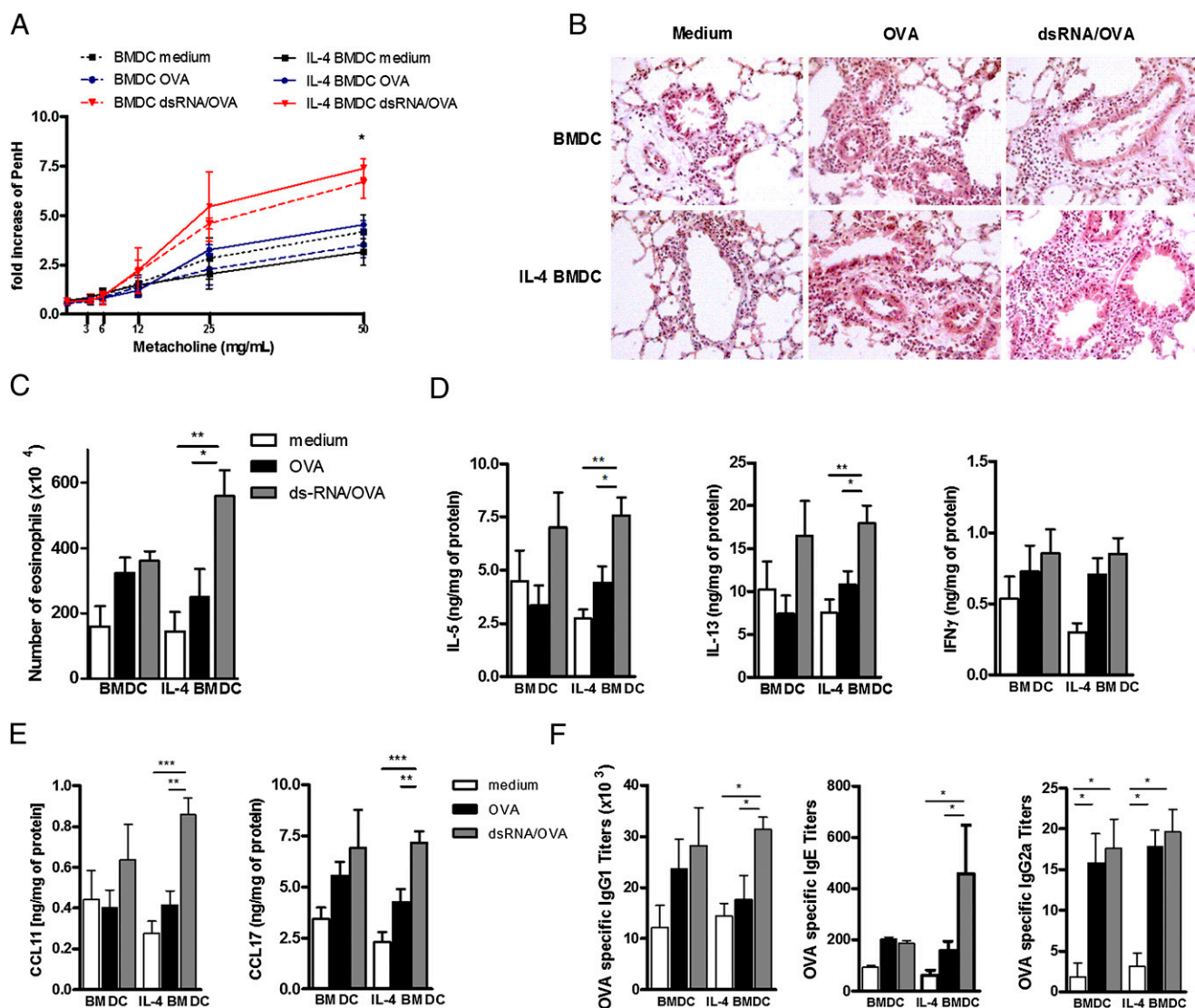


FIGURE 6. Intratracheal administration of BMDCs primed with dsRNA/OVA exacerbates the lung allergic reaction in sensitized mice. **A**, Lung function determined by measurement of PenH of mice receiving intratracheal administration of BMDCs differentiated with GM-CSF (BMDCs) or GM-CSF plus IL-4 (IL-4 BMDCs) and then activated with OVA, with dsRNA/OVA or unstimulated (medium) (* $p < 0.05$ versus mice treated with IL-4 BMDCs plus OVA); **B**, H&E staining of lung sections (original magnification $\times 300$); **C**, eosinophil numbers in BAL; **D** and **E**, cytokine (**D**) and chemokine (**E**) production as determined by ELISA in the protein extract from the lung; and **F**, serum OVA-specific IgG1, IgE, and IgG2a levels. Data are expressed as the mean $\pm$ SD ($n = 5$ mice) and are from one representative experiment out of three. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus mice receiving either BMDCs or IL-4 BMDCs primed with medium or OVA.

with dsRNA significantly enhanced IL-4 and IL-5 production compared with unstimulated and OVA-activated BMDCs. Interestingly, transfer of IL-4 BMDCs induced a higher level of IL-4 compared with BMDCs. In conclusion, our data indicate that transfer of BMDCs exposed to dsRNA/OVA induces exacerbation of the lung allergic reaction and differentiation of BMDCs in the presence of IL-4 amplifies the response.

dsRNA-activated AECs participate in mDC recruitment

Our data revealed that dsRNA exposure induced DC recruitment into the lungs through a TRIF-dependent mechanism. Because AECs are one of the first targets of dsRNA and are involved in DC migration (12, 37, 38), we hypothesized that dsRNA-activated AECs might participate in mDC recruitment through chemokine synthesis. We first analyzed the expression of CCL20, a key chemokine involved in DC migration, in the airway epithelium by immunohistochemistry, 24 h after the last dsRNA/OVA administration (day 17). As

illustrated in Fig. 7A, airway epithelium (arrows) was labeled with anti-CCL20 Ab in OVA- and dsRNA/OVA-treated mice. The percentage of positive AECs was higher in mice that received dsRNA/OVA compared with OVA (71 ± 8 versus $15 \pm 3\%$, respectively). CCL20 increased expression was associated with an augmented number of peribronchial MHC II⁺ cells (Fig. 7A). When compared with OVA alone, dsRNA/OVA administration increased CCL11 and CCL17 synthesis in airway epithelium (33 ± 4 versus $63 \pm 6\%$ for CCL11; 31 ± 6 versus $61 \pm 11\%$ for CCL17, respectively).

We then analyzed in vitro the capacity of dsRNA-activated AECs to produce chemokines and the contribution of the TLR3 pathway in these settings. Activation of TLR3-competent AECs with dsRNA significantly enhanced the secretion of CCL5, CCL20, CXCL10, CCL11, and CCL17 (Fig. 7B). In contrast, dsRNA activation of TLR3-deficient AECs did not trigger the production of these chemokines. Migration of mDCs was then assessed by chemotaxis

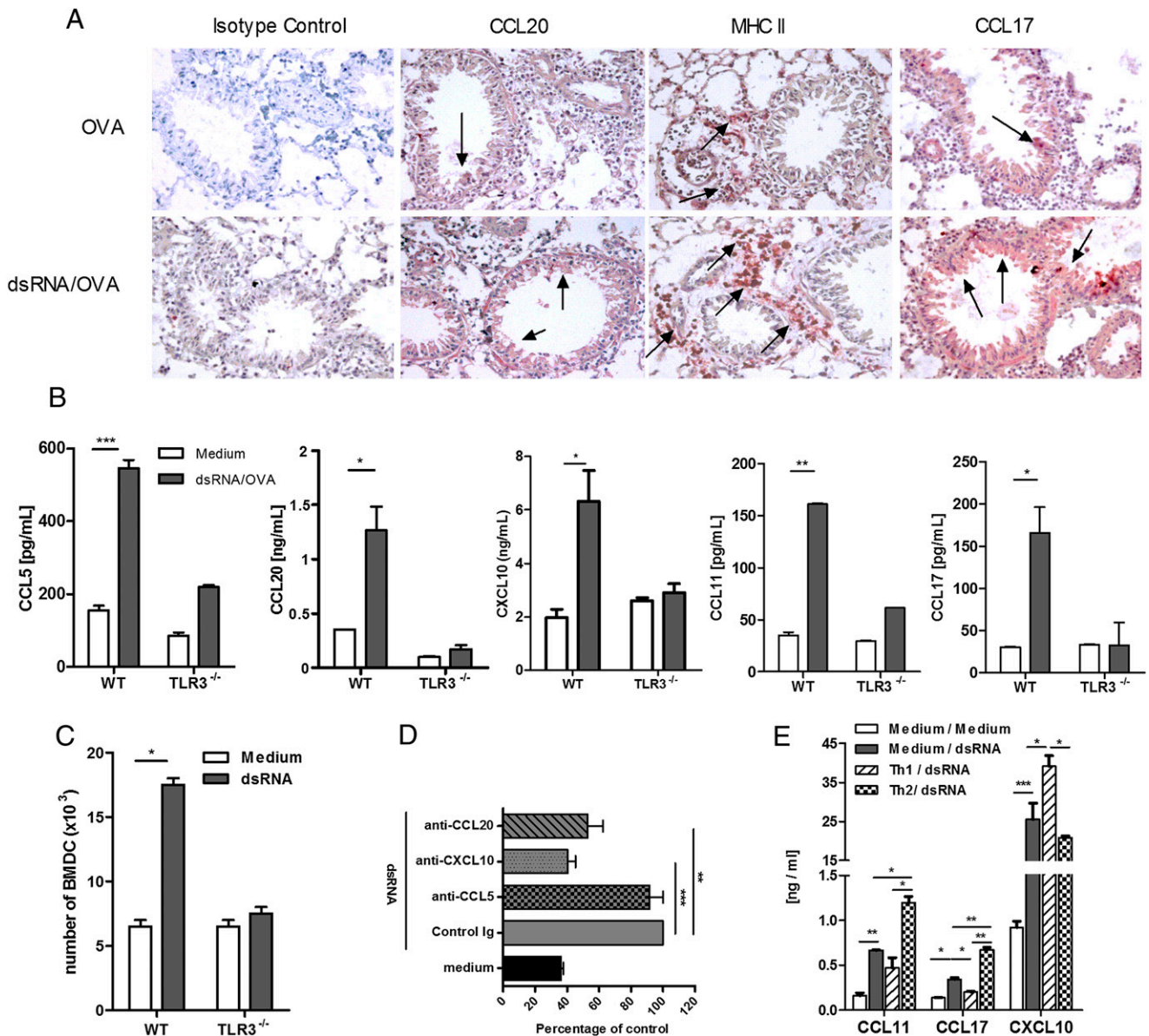


FIGURE 7. dsRNA-activated AECs participate in mDC recruitment through chemokine secretion. *A*, Immunohistochemistry with mAb anti-CCL20, anti-MHC II, and anti-CCL17 or with an isotype control on lung sections from sensitized mice exposed to OVA and dsRNA/OVA (lungs are collected at day 17) (original magnification $\times 300$). Black arrows indicate positive AECs stained in red. Of note, detected positive endothelial cells and fibroblasts are also detected; *B*, dsRNA-induced secretion of CCL5, CCL20, CCL11, CCL17, and CXCL10 by AECs is dependent of TLR3 expression. In vitro, AECs from WT and TLR3^{-/-} mice are activated or not with dsRNA; *C*, chemotactic activity for BMDCs of supernatants from WT and TLR3^{-/-} AECs stimulated with dsRNA; *D*, effect of neutralizing Abs against CCL5, CCL20, and CXCL10 on the BMDC migration induced by dsRNA-stimulated AECs; *E*, in vitro exposure of AEC to Th2 (IL-4 and IL-13) and Th1 (IFN- γ) cytokines modulate dsRNA-induced chemokine production. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus the indicated condition.

assays. Relative to the control, supernatants from dsRNA-activated WT, but not TLR3-deficient AECs, strongly increased the migration of BMDCs (Fig. 7C). Preincubation of supernatants collected from TLR3-expressing AECs with neutralizing Abs showed that CXCL10 and CCL20 are implicated in the migration of BMDCs in response to dsRNA exposure (85 and 70% inhibition, respectively) (Fig. 7D). In contrast, CCL5 was not involved.

Because Th2 cytokine environment modulates BMDC functions (Fig. 6), AECs were sensitized with Th1-type (IFN- γ) or Th2-type (IL-4/IL-13) cytokines, and their responses to dsRNA were analyzed. IFN- γ treatment amplified CXCL10 secretion triggered by dsRNA, whereas IL-4/IL-13 did not modulate its synthesis (Fig. 7E). In contrast, dsRNA-induced production of CCL11 and CCL17 was increased in IL-4/IL-13-, but not IFN- γ -, treated

AECs. Pretreatment of AECs with Th1- and Th2-type cytokines did not affect the capacity of dsRNA-exposed cells both to produce CCL5 and CCL20 and to recruit mDC (data not shown).

These data indicate that, in response to dsRNA, TLR3-competent AECs might be important to promote the recruitment of mDCs and inflammatory cells through chemokine secretion. Although Th2 cytokines did not affect the AEC capacity to recruit DCs, they probably amplify the migration of Th2 cells and eosinophils.

Discussion

Viral infections have been shown to promote allergic asthma exacerbation in the human and mouse systems (16–18). Although experimental models mimicking asthma exacerbation have been reported (19–22), little is known about the cellular and molecular

mechanisms involved in the pathology of this response. To mimic respiratory virus infection, we developed in the current study an experimental model of lung allergic exacerbation, based on the administration of dsRNA. We also demonstrated that the TLR3-TRIF pathway plays a key role in the dsRNA-induced exacerbation of the allergic reaction and that AEC/DC cross-talk may also be involved.

Previous studies have reported that systemic delivery of dsRNA can prevent or suppress experimental asthma through the development of a Th1 response (39). In contrast, local administration of dsRNA during the challenge phase swiftly enhances airway eosinophilia and AHR in sensitized mice (21). Inhalation of low doses of dsRNA (100 ng/mice) during allergen sensitization increased Th2 responses and the airway inflammation, whereas high doses favored Th1 responses (40). We show in this study for the first time that dsRNA instillation in sensitized mice has a long-term effect by inducing exacerbation of the allergic reaction after a secondary allergen challenge. Our unpublished findings reveal that dsRNA given without OVA at days 14–16 does not exacerbate allergic reaction and that coadministration of dsRNA and OVA during the second challenge (days 26–28) had a lower effect on the allergic airway inflammation compared with coadministration at days 14–16 (data not shown). Collectively, it appears that the route and the timing of dsRNA administration are critical for the development of the allergic reaction. In our experimental setting, dsRNA acts as a pro-Th2 adjuvant in sensitized animals and amplifies the allergic reaction through an enhanced Th2-mediated allergen-specific immune response.

Because dsRNA signal is delivered extracellularly in our model, we need to determine how this mimics *in vivo* respiratory viral infection. A large proportion of this signal is expected to be intracellular during viral infections. However, *in vitro* and *in vivo* dsRNA administration has been shown to reproduce most of the proinflammatory effect of pulmonary viruses (41). Our data show that the effect of dsRNA on asthma exacerbation is fully TLR3/TRIF dependent. Interestingly, TLR3 contributes to damages associated with influenza A virus-induced acute pneumonia (35). Having established this we cannot discard the possibility that other innate sensors including RNA helicases, could participate either in a positive or negative manner, in the pathology. Recent reports demonstrated that extracellular delivery of oligonucleotides is efficiently transported toward lysosomal TLR through the mobilization of scavenger receptors (42, 43). Moreover, our model mimics the clinical situation associated with virus-induced asthma exacerbation, including an increase of AHR, eosinophilic airway inflammation, innate immune cell infiltrate, allergen-specific Ig levels, development of Th2 cells, and secretion of Th2-associated chemokines (6, 21, 44). Finally, in mice treated with dsRNA/OVA as well as in animals during respiratory viral infection and during respiratory syncytial virus-enhanced allergic inflammation (45, 46), CD8⁺ T cells represent the majority of T lymphocytes infiltrating lung tissue and draining LNs (Fig. 1E). In conclusion, our model reproduces most of the situation encountered during respiratory viral infection.

Several studies have underlined the involvement of mDCs in the development of Th2 responses and during allergic asthma and the regulatory role of plasmacytoid DCs (1, 47, 48). In this paper, we demonstrated that administration of IL-4-conditioned BMDCs activated with dsRNA reproduced the effect of dsRNA inhalation, whereas BMDCs differentiated without IL-4 have a limited effect. This suggests that Th2 cytokines modify the capacity of mDCs to polarize the T cell response in our model. It is likely that this effect is mediated by an increased CD80 and CD86 expression on BMDCs, costimulatory molecules known to

be involved in the induction of Th2 responses (49). In contrast, no effect on the production of the immunomodulatory cytokines IL-10 and IL-12p70 was observed. This is in line with our finding, revealing augmented expression of CD86 on pulmonary DCs after dsRNA treatment. These data also suggest that mDCs expressing CD86 might be responsible for the effect of dsRNA on the OVA-specific Th2 response and lung inflammation.

To define the mechanisms responsible for mDC mobilization, we evaluated the implication of the AEC, which is the primary target for respiratory virus (50, 51). We and others have shown that the AEC controls the migration and the function of the mDC and therefore is important in mucosal immunity (12, 14). In our model, *in vitro* activation of TLR3-competent AECs by dsRNA enhances mDC migration, suggesting a potential role of airway epithelium in mDC trafficking and the development of asthma exacerbation after dsRNA administration. In parallel, TRIF^{-/-} mice treated with dsRNA/OVA are unable to recruit mDCs and present no exacerbation of the allergic reaction. Notably, our preliminary observations suggest that AECs infected by respiratory viruses display the same functions (data not shown). As previously described with a TLR2 ligand (12), we report that CCL20 and CXCL10 play an essential role in mDC migration. In agreement with this, airway epithelium expressed a higher level of CCL20 after dsRNA/OVA administration relative to mice challenged with OVA alone. This enhanced production was associated with a heightened infiltration of MHC II⁺ cells, such as DCs, in the peribronchial area. Our data also show that AEC exposure to Th2 cytokines did not amplify mDC recruitment. This result is in accordance with the lack of effect of IL-4 and IL-13 on the production of these chemokines. Moreover, because these chemokines are also produced by other cells, including smooth muscle cells, we cannot exclude the implication of these cells in the recruitment of lung DCs.

Airway epithelium is also involved in the direct control of lung allergic inflammation through CCL11 and CCL17 synthesis (52). Indeed, *in vivo* dsRNA/OVA administration enhances the production of CCL11 and CCL17 by airway epithelium. In addition, *in vitro* AEC pretreatment with Th2 cytokines enhances the dsRNA-induced synthesis of CCL11 and CCL17. Thus, through their ability to recruit eosinophils and Th2 lymphocytes (53), AEC-derived CCL11 and CCL17 may be important in the exacerbation of allergic reaction.

In summary, our findings reveal for the first time that dsRNA exacerbates lung allergic reaction through the TLR3/TRIF pathway. Our data also pinpoint a crucial role for airway epithelium in asthma exacerbation, an effect likely to be because of DCs and inflammatory cell mobilization. This observation might be helpful to propose new therapeutic approaches to better control virus-induced asthma exacerbation in the future.

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Disclosures

The authors have no financial conflicts of interest.

References

- Herrick, C. A., and K. Bottomly. 2003. To respond or not to respond: T cells in allergic asthma. *Nat. Rev. Immunol.* 3: 405–412.
- Kay, A. B., S. Phipps, and D. S. Robinson. 2004. A role for eosinophils in airway remodelling in asthma. *Trends Immunol.* 25: 477–482.
- Larché, M., D. S. Robinson, and A. B. Kay. 2003. The role of T lymphocytes in the pathogenesis of asthma. *J. Allergy Clin. Immunol.* 111: 450–463, quiz 464.

4. Wills-Karp, M. 1999. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol.* 17: 255–281.
5. Cohn, L. S., A. Elias, and G. L. Chupp. 2004. Asthma: mechanisms of disease persistence and progression. *Annu. Rev. Immunol.* 22: 789–815.
6. Robinson, D. S., Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A. M. Bentley, C. Corrigan, S. R. Durham, and A. B. Kay. 1992. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326: 298–304.
7. Coyle, A. J., F. Erard, C. Bertrand, S. Walti, H. Pircher, and G. Le Gros. 1995. Virus-specific CD8⁺ cells can switch to interleukin 5 production and induce airway eosinophilia. *J. Exp. Med.* 181: 1229–1233.
8. Foster, P. S., S. P. Hogan, A. I. Ramsay, K. I. Matthaei, and I. G. Young. 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183: 195–201.
9. Lambrecht, B. N., M. De Veerman, A. J. Coyle, J. C. Gutierrez-Ramos, K. Thielemans, and R. A. Pauwels. 2000. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J. Clin. Invest.* 106: 551–559.
10. Lambrecht, B. N., B. Salomon, D. Klatzmann, and R. A. Pauwels. 1998. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J. Immunol.* 160: 4090–4097.
11. Pichavant, M., A. S. Charbonnier, S. Taront, A. Brichet, B. Wallaert, J. Pestel, A. B. Tonnel, and P. Gosset. 2005. Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment. *J. Allergy Clin. Immunol.* 115: 771–778.
12. Pichavant, M., S. Taront, P. Jeannin, L. Breuilh, A. S. Charbonnier, C. Spriet, C. Fournieu, N. Corvaia, L. Hélot, A. Brichet, et al. 2006. Impact of bronchial epithelium on dendritic cell migration and function: modulation by the bacterial motif KpOmpA. *J. Immunol.* 177: 5912–5919.
13. Reibman, J., Y. Hsu, L. C. Chen, B. Bleck, and T. Gordon. 2003. Airway epithelial cells release MIP-3α/α/CCL20 in response to cytokines and ambient particulate matter. *Am. J. Respir. Cell Mol. Biol.* 28: 648–654.
14. Roggen, E. L., M. Lindstedt, C. Borrebaeck, and G. R. Verheyen. 2006. Interactions between dendritic cells and epithelial cells in allergic disease. *Toxicol. Lett.* 162: 71–82.
15. Hammad, H., M. Chieppa, F. Perros, M. A. Willart, R. N. Germain, and B. N. Lambrecht. 2009. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat. Med.* 15: 410–416.
16. Holt, P. G., and P. D. Sly. 2002. Interactions between RSV infection, asthma, and atopy: unraveling the complexities. *J. Exp. Med.* 196: 1271–1275.
17. Minor, T. E., E. C. Dick, J. W. Baker, J. J. Ouellette, M. Cohen, and C. E. Reed. 1976. Rhinovirus and influenza type A infections as precipitants of asthma. *Am. Rev. Respir. Dis.* 113: 149–153.
18. Thumerelle, C., A. Deschildre, C. Bouquillon, C. Santos, A. Sardet, M. Scalbert, L. Delbecq, P. Debray, A. Dewilde, D. Turck, and F. Leclerc. 2003. Role of viruses and atypical bacteria in exacerbations of asthma in hospitalized children: a prospective study in the Nord-Pas de Calais region (France). *Pediatr. Pulmonol.* 35: 75–82.
19. Tripp, R. A., D. Moore, and L. J. Anderson. 2000. TH(1)- and TH(2)-type cytokine expression by activated T lymphocytes from the lung and spleen during the inflammatory response to respiratory syncytial virus. *Cytokine* 12: 801–807.
20. Barends, M., M. Van Oosten, C. G. De Rond, J. A. Dormans, A. D. Osterhaus, H. J. Neijens, and T. G. Kimman. 2004. Timing of infection and prior immunization with respiratory syncytial virus (RSV) in RSV-enhanced allergic inflammation. *J. Infect. Dis.* 189: 1866–1872.
21. Bartlett, N. W., R. P. Walton, M. R. Edwards, J. Anisenco, G. Caramori, J. Zhu, N. Glanville, K. J. Choy, P. Jourdan, J. Burnet, et al. 2008. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat. Med.* 14: 199–204.
22. Dahl, M. E., K. Dabbagh, D. Liggett, S. Kim, and D. B. Lewis. 2004. Viral-induced T helper type 1 responses enhance allergic disease by effects on lung dendritic cells. *Nat. Immunol.* 5: 337–343.
23. Reynolds, H. Y. 2002. Modulating airway defenses against microbes. *Curr. Opin. Pulm. Med.* 8: 154–165.
24. Gordon, S. 2002. Pattern recognition receptors: doubling up for the innate immune response. *Cell* 111: 927–930.
25. Alexopoulou, L., A. C. Holt, R. Medzhitov, and R. A. Flavell. 2001. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. *Nature* 413: 732–738.
26. Atmar, R. L., E. Guy, K. K. Guntupalli, J. L. Zimmerman, V. D. Bandi, B. D. Baxter, and S. B. Greenberg. 1998. Respiratory tract viral infections in inner-city asthmatic adults. *Arch. Intern. Med.* 158: 2453–2459.
27. Doyle, S. E., R. O'Connell, S. A. Vaidya, E. K. Chow, K. Yee, and G. Cheng. 2003. Toll-like receptor 3 mediates a more potent antiviral response than Toll-like receptor 4. *J. Immunol.* 170: 3565–3571.
28. Jiang, Z., M. Zamanian-Daryoush, H. Nie, A. M. Silva, B. R. Williams, and X. Li. 2003. Poly(I:C)-induced Toll-like receptor 3 (TLR3)-mediated activation of NFκB and MAP kinase is through an interleukin-1 receptor-associated kinase (IRAK)-independent pathway employing the signaling components TLR3-TRAF6-TAK1-TAB2-PKR. *J. Biol. Chem.* 278: 16713–16719.
29. Hewson, C. A., A. Jardine, M. R. Edwards, V. Laza-Stanca, and S. L. Johnston. 2005. Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells. *J. Virol.* 79: 12273–12279.
30. Le Goffic, R., J. Pothlichet, D. Vitour, T. Fujita, E. Meurs, M. Chignard, and M. Si-Tahar. 2007. Cutting edge: influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. *J. Immunol.* 178: 3368–3372.
31. Yamamoto, M., S. Sato, H. Hemmi, K. Hoshino, T. Kaisho, H. Sanjo, O. Takeuchi, M. Sugiyama, M. Okabe, K. Takeda, and S. Akira. 2003. Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. *Science* 301: 640–643.
32. Yamamoto, M., S. Sato, K. Mori, K. Hoshino, O. Takeuchi, K. Takeda, and S. Akira. 2002. Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-β promoter in the Toll-like receptor signaling. *J. Immunol.* 169: 6668–6672.
33. Desmet, C., P. Gosset, B. Pajak, D. Cataldo, M. Bentires-Alj, P. Lekeux, and F. Bureau. 2004. Selective blockade of NF-κB activity in airway immune cells inhibits the effector phase of experimental asthma. *J. Immunol.* 173: 5766–5775.
34. Guillot, L., R. Le Goffic, S. Bloch, N. Escricou, S. Akira, M. Chignard, and M. Si-Tahar. 2005. Involvement of Toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus. *J. Biol. Chem.* 280: 5571–5580.
35. Le Goffic, R., V. Balloy, M. Lagranderie, L. Alexopoulou, N. Escricou, R. Flavell, M. Chignard, and M. Si-Tahar. 2006. Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathog.* 2: e53.
36. Lambrecht, B. N., R. A. Peleman, G. R. Bullock, and R. A. Pauwels. 2000. Sensitization to inhaled antigen by intratracheal instillation of dendritic cells. *Clin. Exp. Allergy* 30: 214–224.
37. Rennard, S. I., D. J. Romberger, J. H. Sisson, S. G. Von Essen, I. Rubinstein, R. A. Robbins, and J. R. Spurzem. 1994. Airway epithelial cells: functional roles in airway disease. *Am. J. Respir. Crit. Care Med.* 150: S27–S30.
38. Sha, Q., A. Q. Truong-Tran, J. R. Plitt, L. A. Beck, and R. P. Schleimer. 2004. Activation of airway epithelial cells by Toll-like receptor agonists. *Am. J. Respir. Cell Mol. Biol.* 31: 358–364.
39. Sel, S., M. Wegmann, S. Sel, S. Bauer, H. Garn, G. Alber, and H. Renz. 2007. Immunomodulatory effects of viral TLR ligands on experimental asthma depend on the additive effects of IL-12 and IL-10. *J. Immunol.* 178: 7805–7813.
40. Jeon, S. G., S. Y. Oh, H. K. Park, Y. S. Kim, E. J. Shim, H. S. Lee, M. H. Oh, B. Bang, E. Y. Chun, S. H. Kim, et al. 2007. TH2 and TH1 lung inflammation induced by airway allergen sensitization with low and high doses of double-stranded RNA. *J. Allergy Clin. Immunol.* 120: 803–812.
41. Stowell, N. C., J. Seidman, H. A. Raymond, K. A. Smalley, R. J. Lamb, D. D. Egenolf, P. J. Bugelski, L. A. Murray, P. A. Marsters, R. A. Bunting, et al. 2009. Long-term activation of TLR3 by poly(I:C) induces inflammation and impairs lung function in mice. *Respir. Res.* 10: 43.
42. Gursel, M., I. Gursel, H. S. Mostowski, and D. M. Klinman. 2006. CXCL16 influences the nature and specificity of CpG-induced immune activation. *J. Immunol.* 177: 1575–1580.
43. Limmon, G. V., M. Arredouani, K. L. McCann, R. A. Corn Minor, L. Kobzik, and F. Imani. 2008. Scavenger receptor class-A is a novel cell surface receptor for double-stranded RNA. *FASEB J.* 22: 159–167.
44. Hansbro, N. G., J. C. Horvat, P. A. Wark, and P. M. Hansbro. 2008. Understanding the mechanisms of viral induced asthma: new therapeutic directions. *Pharmacol. Ther.* 117: 313–353.
45. Hikono, H., J. E. Kohlmeier, K. H. Ely, I. Scott, A. D. Roberts, M. A. Blackman, and D. L. Woodland. 2006. T-cell memory and recall responses to respiratory virus infections. *Immunol. Rev.* 211: 119–132.
46. Jiang, X. B., Z. D. Wang, Y. Zhu, X. L. Zhang, X. F. Cui, K. Yao, and K. S. Yin. 2009. Inhibition of CD8⁺ T lymphocytes attenuates respiratory syncytial virus-enhanced allergic inflammation. *Respiration* 77: 76–84.
47. Lambrecht, B. N., and H. Hammad. 2003. Taking our breath away: dendritic cells in the pathogenesis of asthma. *Nat. Rev. Immunol.* 3: 994–1003.
48. Vermaelen, K., and R. Pauwels. 2003. Accelerated airway dendritic cell maturation, trafficking, and elimination in a mouse model of asthma. *Am. J. Respir. Cell Mol. Biol.* 29: 405–409.
49. van Rij, L. S., N. Vos, M. Willart, A. Kleinjan, A. J. Coyle, H. C. Hoogsteden, and B. N. Lambrecht. 2004. Essential role of dendritic cell CD80/CD86 costimulation in the induction, but not reactivation, of TH2 effector responses in a mouse model of asthma. *J. Allergy Clin. Immunol.* 114: 166–173.
50. Message, S. D., and S. L. Johnston. 2004. Host defense function of the airway epithelium in health and disease: clinical background. *J. Leukoc. Biol.* 75: 5–17.
51. Tamura, S., and T. Kurata. 2004. Defense mechanisms against influenza virus infection in the respiratory tract mucosa. *Jpn. J. Infect. Dis.* 57: 236–247.
52. Holgate, S. T., P. M. Lackie, D. E. Davies, W. R. Roche, and A. F. Walls. 1999. The bronchial epithelium as a key regulator of airway inflammation and remodelling in asthma. *Clin. Exp. Allergy* 29(Suppl. 2): 90–95.
53. Tsuji, K., S. Yamamoto, W. Ou, N. Nishi, I. Kobayashi, M. Zaitu, E. Muro, Y. Sadakane, T. Ichimaru, and Y. Hamasaki. 2005. dsRNA enhances eotaxin-3 production through interleukin-4 receptor upregulation in airway epithelial cells. *Eur. Respir. J.* 26: 795–803.