Caspase-11 regulates lung inflammation in response to house dust mites

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Abstract

Asthma is an inflammatory lung disorder characterized by mucus hypersecretion, cellular infiltration, and bronchial hyper-responsiveness. House dust mites (HDM) are the most prevalent cause of allergic sensitization. Canonical and noncanonical inflammasomes are multiprotein complexes that assemble in response to pathogen or danger-associated molecular patterns (PAMPs or DAMPs). Murine caspase-11 engages the noncanonical inflammasome. We addressed the role of caspase-11 in mediating host responses to HDM and subsequent allergic inflammation using *caspase-11*$^{-/-}$ mice, which lack caspase-11 while express caspase-1. We found that HDM induce caspase-11 expression *in vitro*. The presence of IL-4 and IL-13 promotes caspase-11 expression. Additionally, *caspase-11*$^{-/-}$ macrophages show reduced release of (KC, IL-6 and IL-12) cytokines, and express lower levels of costimulatory molecules (e.g., CD40, CD86 and MHCII) in response to HDM stimulation. Notably, HDM sensitization of *caspase-11*$^{-/-}$ mice resulted in similar levels of IgE responses and hypothermia in response to nasal HDM challenge compared to WT. However, analysis of cell numbers and cytokines in bronchiolar alveolar fluid (BALF), as well as histological lung tissue showed altered inflammatory responses and reduced neutrophilia in the airways of the *caspase-11*$^{-/-}$ mice. These findings indicate that caspase-11 regulates airway inflammation in response to HDM exposure.

Introduction

Asthma is a long-term recurring inflammatory lung disorder characterized by mucus hypersecretion, cellular infiltration, chronic airway inflammation and bronchial hyper-responsiveness (Platts-Mills and Chapman 1987, Arlian and Platts-Mills 2001, Thomas 2012). Different forms of asthma can coexist in some patients including atopic (allergic, Th2-dependent), and non-atopic (non-allergic, Th2-independent) forms. These forms are initiated and regulated by multiple genetic and environmental factors, such as HDM allergens.

House dust mites, such as *Dermatophagoides pteronyssinus*, are an unsurpassed cause of atopic sensitization and the major cause of allergic asthma worldwide (Calderon, Linneberg et al. 2015, Sanchez-Borges, Fernandez-Caldas et al. 2017). A battery of *D. pteronyssinus* allergens, proteases, bacterial lipopolysaccharide (LPS), and chitin from the mite exoskeleton are present in fecal pellets and decaying mite materials and have potent sensitizing capacities (Thomas, Hales et al. 2010, Bordas-Le Floch, Le Mignon et al. 2017). Proteases-dependent effect of mite allergens is associated with breaching the epithelial layer, breaking tight junctions and stimulating protease-activated receptors. These events lead to increased epithelium permeability and production of chemokines and cytokines, which recruits antigen presenting cells (APCs) into the epithelial layers, thus promoting airway inflammation and remodelling (Asokananthan, Graham et al. 2002, Kato, Takai et al. 2009, Jacquet 2011, Jacquet 2011, Kubo 2017). Even though $CD4^+$ Th2 cells orchestrate the HDM allergic response via production of IgE against mite allergens, promoting inflammation and lung remodelling (Na, Cho et al. 2016), it is now recognized that the innate immune system plays a fundamental role in initiating and shaping the allergic

response by programming and maintaining Th2-biased adaptive immunity in response to HDM allergens and their contaminants (Hammad, Chieppa et al. 2009, Willart and Lambrecht 2009).

Innate immune system activation is mediated through pattern recognition receptors (PRRs), which sense PAMPs and DAMPs (Janeway and Medzhitov 2002, Kawai and Akira 2010). The PRR families include intracellular NOD-like receptors (NLRs) and extracellular Toll-like receptors (TLRs) (Kawai and Akira 2010). Sensing PAMPs or DAMPs by the NLR protein family, such as NLRP3 or NLRC4, mediates the assembly of inflammasomes. Downstream of inflammasome signaling, IL-1β and IL-18 are cleaved by active caspase-1 and delineates the canonical inflammasome pathway (Lamkanfi and Dixit 2014, Yang, Zhao et al. 2015, Broz and Dixit 2016). In addition to canonical inflammasome activation, caspase-11 mediates non-canonical inflammasome activation in response to cytosolic LPS (Kayagaki, Warming et al. 2011).

Caspase-11 in mice or (caspase-4/5 in humans) belong to the family of inflammatory caspases and exhibits (46%) similarity to caspase-1 (Faucheu, Diu et al. 1995, Kamens, Paskind et al. 1995, Munday, Vaillancourt et al. 1995, Faucheu, Blanchet et al. 1996, Wang, Miura et al. 1998, Lin, Choi et al. 2000, Lamkanfı, Declercq et al. 2002, Martinon and Tschopp 2004). It has been demonstrated that *caspase-11*$^{-/-}$ but not *caspase-1*$^{-/-}$ mice are resistant to septic shock mediated by injection of lethal doses of LPS (Kayagaki, Warming et al. 2011, Kayagaki, Wong et al. 2013). Caspase-11 expression is induced via multiple TLR stimulants and interferons (Kayagaki, Warming et al. 2011, Kayagaki, Wong et al. 2013). In this regard, extracellular LPS and cytokines primarily serve as a priming signal for expression of caspase-11 and other inflammasome components. Furthermore, intracellular LPS triggers caspase-11-dependent inflammasome activation in the cytoplasm independently of TLR4 (Bauernfeind, Horvath et al. 2009, Kayagaki, Warming et al. 2011, Hagar, Powell et al. 2013, Kayagaki, Wong et al. 2013, Caution, Young et al. 2019).

In this study, we use caspase-*11*$^{-/-}$ mice and HDM-mediated allergic inflammation model to delineate the role of caspase-11, *in vivo*. We also demonstrate that the inflammatory environment of asthma, specifically IL-4 and IL-13 cytokines, promote caspase-11 expression. Furthermore, *caspase-11*$^{-/-}$ macrophages produce lower amounts of (KC, IL-6, and IL-12), and express lower levels of costimulatory molecules (e.g., CD40, CD86 and MHCII) in response to HDM stimulation. *In vivo* data show that HDM induce caspase-11 expression in WT mice. Furthermore, HDM promote differential inflammation and cellular infiltration with lessened neutrophilia in the *caspase-11*$^{-/-}$ mice. Therefore, caspase-11 promotes inflammation and neutrophil trafficking in response to HDM exposure.

Results

HDM promote caspase-11 expression in macrophages derived from WT mice.

House dust mites and their components (allergens, proteases, chitin, microbial LPS and ligands originating from mite-associated compounds) are activators of innate and adaptive immunity (Gregory

and Lloyd 2011). Since caspase-11 is only expressed upon induction with pro-inflammatory stimuli (Schauvliege, Vanrobaeys et al. 2002), we tested whether HDM induce caspase-11 expression *in vitro* using primary bone marrow derived macrophages (BMDMs) from WT C57BL/6 mice. In contrast to freshly isolated lung macrophages, dendritic cells or epithelial cells, BMDMs are not activated or primed. Therefore their response will be directed only to HDM stimulation. The BMDM were treated with different concentrations of HDM for 4 or 24 h, and caspase-11 expression was detected by Western blotting (Fig. 1A). Our data show that caspase-11 expression is induced 4 h post-HDM at all used concentrations, and stays active up to 24 hours post-treatment.

Allergy-associated cytokines promote caspase-11 expression in macrophages derived from WT mice.

Macrophages are classified into classically (M1) and alternatively activated macrophages (M2 or AAM), which reflect the T helper (Th1/Th2) subsets (Gordon 2003, Sica and Mantovani 2012). M1 macrophages promote inflammation, whereas M2 macrophages are critical for inflammation resolution (Awad, Assrawi et al. 2017). To determine if the inflammatory environment of asthma, and more specifically IL-4 and IL-13 cytokines, promote caspase-11 expression, we treated macrophages with IL-4 or IL-13 and examined caspase-11 expression by Western blotting. Our data demonstrate that macrophage treatment with IL-4 or IL-13 induces caspase-11 expression at 1, 4, 6 and 24 h post-treatment, similar to the treatment with other inflammatory stimuli like IL-1β, IL-1α and LPS (Fig. 1B & C).

We sought to identify the mediator of caspase-11 induction in response to IL-4 and IL-13 treatment. Caspase-11 is highly inducible with a 5` upstream regulatory promoter region comprised of multiple NF-κB binding sites and one STAT binding site (Schauvliege, Vanrobaeys et al. 2002). Therefore, we tested if IL-4 or IL-13-induced expression of caspase-11 leads to STAT6 phosphorylation. Our data demonstrate that macrophages treatment with IL-4 and IL-13 induced phosphorylation of STAT6 in both WT and *caspase-11*$^{-/-}$ (Fig. 1D & E). Notably, STAT6 phosphorylation was not observed following treatment with IL-1β (Fig. 1D & E). Similar total STAT6 was detected in all the samples (Fig. 1E). Therefore, the inflammatory environment of asthma, specifically IL-4 and IL-13 cytokines, induces caspase-11 expression and leads to STAT6 phosphorylation.

Macrophages derived from caspase-11$^{-/-}$ **mice show reduced proinflammatory cytokine responses and reduced costimulatory molecule expression in response to HDM treatment.**

IL-1β is a pivotal cytokine in asthma pathogenesis. Thus, it plays a central role in HDM-mediated allergic inflammation (Jacquet 2011, Sundaram, Mitra et al. 2015), and its presence often designates the activation of the inflammasome (Kayagaki, Warming et al. 2011, Broz, Ruby et al. 2012, Gurung, Malireddi et al. 2012, Rathinam, Vanaja et al. 2012). Therefore, we determined the role of caspase-11 in the production of IL-1β by treating WT or *caspase-11*$^{-/-}$ macrophages with 100 μg/mL of HDM. In contrast to WT, *caspase-11*$^{-/-}$ macrophages showed significant decrease in the IL-1β release (Fig. 2A). Therefore, HDM induces caspase-11 expression and increased IL-1β release in macrophages derived from WT mice.

HDM elicit a strong immune response via promoting proinflammatory and anti-inflammatory cytokines. IL-4 is released and upregulated in asthma (Kay 2001, Dullaers, De Bruyne et al. 2012). Even though, HDM or IL-4 both induced caspase-11 expression, we hypothesized that caspase-11 response would be different in an environment containing the allergen only (which models the priming phase), or in asthma-like environment where both allergen and IL-4 are present (which represent the effector phase). To examine the role of caspase-11 in response to these environments, we treated macrophages with HDM to model the priming phase or polarized BMDMs toward M2 by stimulating macrophages with IL-4 overnight followed by treatment with HDM + IL-4 for 24 h, indicating the effector phase. Our data demonstrate that *caspase-11*$^{-/-}$ macrophages release significantly less KC, IL-6 and IL-12 at 24 h post-HDM treatment when compared to their WT counterparts (Fig. 2B, C, & D). Treatment with IL-4 in the presence of HDM yield the same trend of responses with significant decrease in the levels of released KC, IL-6 and IL-12 following IL-4 treatment plus 100 µg HDM in *caspase-11*$^{-/-}$ macrophages (Fig. 2B, C, & D). In contrast to KC, treatment with IL-4 + HDM induced a significant increase in the level of IL-6 at 100 µg/mL of HDM in both WT and *caspase-11*$^{-/-}$ macrophages. However, the level of IL-6 was still significantly reduced in *caspase-11*$^{-/-}$ compared to WT macrophages (Fig. 2C). Therefore, our data demonstrate reduced release of pro-inflammatory cytokines (KC, IL-6 and IL-12) from *caspase-11*$^{-/-}$ macrophages as compared to WT in response to HDM. Furthermore, when we simulated the allergic environment of asthma by treating cells with HDM and IL-4, *caspase-11*$^{-/-}$ macrophages show reduced release of (KC, IL-6 and IL-12). Therefore, our data demonstrate reduced cytokines release by *caspase-11*$^{-/-}$ derived macrophages in an environment similar to asthma.

Macrophages are involved in antigen presentation to immune effector cells, providing two specific signals to achieve optimum activation of T cells (McAdam, Schweitzer et al. 1998). The CD28 and CD40L molecules on T cells are known to interact, respectively, with costimulatory-molecules CD80/CD86, and CD40 on antigen presenting cells (APCs) (Greenfield, Nguyen et al. 1998, Grewal and Flavell 1998). Therefore, we used flow cytometry to examine CD86 and CD40 expression, 24 h post-HDM, or post-HDM + IL-4 treatment in WT and *caspase-11*$^{-/-}$ macrophages. *Caspase-11*$^{-/-}$ macrophages showed significantly reduced levels of CD86, CD40 and MHCII 24 h post-HDM + IL-4 stimulation (Fig. 2E & F). In asthmatic animals, the M2 phenotype of macrophages is associated with high level of arginase-1 and family proteins chitinase-like Ym1 (Bhatia, Fei et al. 2011, Hong, Chung et al. 2014, Zhong, Yang et al. 2014). Under HDM + IL-4 stimulation, *caspase-11*$^{-/-}$ macrophages expressed higher levels of *arginase-1* and *Ym1* but lower levels of the M1 marker (*Cxcl10*), when compared to macrophages treated with HDM only (Fig. 2G, H & I). Together, *caspase-11*$^{-/-}$ macrophages express lower levels of costimulatory molecules and higher levels of arginase-1 and Ym1 associated with M2 phenotype.

HDM sensitization and challenge promotes differential allergic inflammation and antibody responses in WT and caspase-11$^{-/-}$ mice.

Recently, an acute model of HDM-mediated experimental allergic lung inflammation was used to delineate the role of caspase-1 in asthma (Madouri, Guillou et al. 2015). However, mice used in these

studies lack both caspase 1 and caspase 11 and thus, it is not clear if the phenotype observed is due to the lack of *caspase-1*, *caspase-11*, or both. Since we detected caspase-11 expression and differential release of inflammatory cytokines in response to HDM *in vitro*, we established a mouse model to study the role of caspase-11 in HDM-mediated inflammation. WT mice were sensitized with alum + 100 µg HDM on day 0 and 7 intra-peritoneally. Then mice were challenged with 13 doses of HDM intranasally (25 µg). Control WT mice were mock sensitized and challenged with Phosphate-buffered saline (PBS) (Supplementary Fig. 1A). Optimization experiments were performed initially to determine the number of HDM challenges needed to provoke asthma symptoms. Self-isolation, low activity and drop in body temperature after each challenge were used as basic measurements to evaluate signs of allergy (Supplementary Fig. 1B). Our data show that HDM administration mediatesd successive and daily drop in body temperatures, which confirmed the validity of our model. Moreover, lungs derived from mice challenged with HDM showed an increase resistance to increasing concentrations of methacholine (Supplementary Fig. 1C). Allergy was evaluated by measuring total level of IgE in the serum and BAL fluid of WT mice. HDM-challenged mice show elevated level of total IgE in sera and BAL fluids as compared to the PBS control (Supplementary Fig. 1D & E). Our results show a significant increase in the HDM-specific IgG1 and IgG2A isotypes in the serum (Supplementary Fig. 1F & G). We further demonstrate that HDM induce caspase-11 expression *in vivo* in WT mice (Supplementary Fig. 1H). Therefore, we successfully induced systemic and lung inflammation in HDM- sensitized and challenged WT mice.

To investigate the role of caspase-11 in mediating allergic lung inflammation, *caspase-11*$^{-/-}$ and WT mice were sensitized with alum + 100 µg HDM on day 0 and 7 intra-peritoneally. Then, mice were challenged with 15 doses of HDM intranasally (25 µg) (Fig. 3A). Both WT and *caspase-11*$^{-/-}$ mice showed signs of allergy, which were consistent with low activity and self-isolation. *Caspase-11*$^{-/-}$ mice showed significantly higher drop in body temperature on challenges 9, 13 and 14 as compared to WT mice. However, no significant difference was seen after challenge 15 which corresponds to day 35 (Fig. 3B). Further, markers of allergy were evaluated in sera and BAL fluid from WT and *caspase-11*$^{-/-}$ mice challenged with HDM. IgE antibody is normally present at low level in the plasma and is mainly produced by plasma cells in mucosal-associated lymphoid tissues. Atopic conditions, such as asthma, elevate serum levels of total IgE that drives the disease. Continuous exposure to HDM allergens lead to continuous production of HDM-specific IgE as well as continued maintenance of allergic inflammation in the airways (Wahn, Lau et al. 1997, Thomas, Hales et al. 2010). Therefore, we examined IgE levels in the sera of WT and *caspase-11*$^{-/-}$ mice before sensitization (day 0), after sensitization with HDM and alum (day 14), day 28, and 24 h after the last challenge (day 35). Our data show that sera and BAL fluid of WT and *caspase-11*$^{-/-}$ mice exhibit similar total IgE levels, (Fig. 3C, D). Furthermore, sera-derived from WT and *caspase-11*$^{-/-}$ had similar levels of HDM-specific IgG1 on day 14, 28 and 35 (Fig. 3G). However, BAL fluid derived from *caspase-11*$^{-/-}$ show significantly higher levels of total IgA on day 28 and 35 (Fig. 3F). There was no difference in HDM-specific IgA titers in the BAL fluid on day 35 (Fig. 3E). Our data demonstrate that BAL derived from *caspase-11*$^{-/-}$ mice show elevated level of IgA as compared to WT. Nevertheless, no significant differences were seen in total IgE, lung functions or body temperature.

Therefore, HDM promotes differential allergic inflammation and antibody responses in WT and *caspase-11*$^{-/-}$ mice.

WT and caspase-11$^{-/-}$ **mice show differential cellular infiltration in their BAL fluid in response to HDM.**

Development of allergic responses requires activation of mast cells, basophils, and eosinophils (Wambre, James et al. 2012). Therefore, we examined infiltration into the BAL fluid of WT and *caspase-11*$^{-/-}$ mice on day 35. Our data show a significant decrease in the total cellular infiltration into the BAL fluid of *caspase-11*$^{-/-}$ mice compared to WT (Fig. 4A). Cells in the BAL fluid were immobilized on cytospin slides, fixed and stained to visualize specific cell populations. BAL from *caspase-11*$^{-/-}$ mice show significant increase in the number of eosinophils (Fig. 4B). BAL fluids were also analyzed by flow cytometry. Our data demonstrate that *caspase-11*$^{-/-}$ mice exhibit a significant increase in eosinophils (CD11b^{+}SiglecF^{+}), total T cells (CD3^{+}), T helper cells (CD3^{+}CD8^{-}) (Fig. 4F, I & K). However, no significant difference was observed in myeloid cells (CD11b^{+}), alveolar macrophages (CD11c^{+}F480^{+}), neutrophils (CD11b^{+}Ly6G^{+}), NK cells (CD49b^{+}), B cells (CD19^{+}), cytotoxic T cells (CD3^{+}CD8^{+}) and mast cells (Ckit^{+}) (Fig. 4C, D, E, G, H, J & L) in BAL fluid. Therefore, *caspase-11*$^{-/-}$ mice show differential cellular infiltration in their BAL fluid as compared to WT in response to HDM.

Caspase-11 $^{-/-}$ **mice show reduced histologic signs of lung inflammation but similar airway resistance and mucus production compared to WT mice**.

Asthma is often sustained by allergic sensitization, which leads to bronchial hyper-responsiveness and acute bronchoconstriction in response to specific and non-specific triggers. HDM cause bronchoconstriction in asthma patients and induce an inflammatory response in the lungs due to the release of cytokines, chemokines and additional mediators. Lungs of both WT and *caspase-11*$^{-/-}$ mice were similarly resistant to increasing concentrations of methacholine measured by Flexivent (Fig. 5A). To determine the effect of chronic HDM challenge on lung pathology in mice, lung tissues were collected 24 h after the last challenge. Lung sections were separately stained with H&E and PAS. HDM challenge for 35 days caused significant reduction in inflammatory cell infiltration in *caspase-11*$^{-/-}$ lungs as compared to WT but similar mucus production (Fig. 5B). Scored inflammation in lung sections shows that *caspase-11*$^{-/-}$ lungs exhibit reduced neutrophils, macrophages and lymphocytes as compared to WT (Fig. 5C). Mucus hypersecretion was examined by measuring the expression level of *muc5ac* in lungs of WT and *caspase-11*$^{-/-}$ mice by RT-qPCR (Fig. 5D). WT and *caspase-11*$^{-/-}$ mice express similar levels of *muc5ac* in their lungs. These data demonstrate that *caspase-11*$^{-/-}$ lungs show reduced infiltration of inflammatory cells into the lung tissue.

Serum and BAL from caspase-11$^{-/-}$ **mice show differential levels of Th1 and Th2 cytokines compared to WT**.

HDM allergens initiate and sustain allergic inflammation. T cells play a role in the initiation and perpetuation of inflammation (Ricci, Rossi et al. 1993, Woodfolk 2007). In particular, Th2 cells have been identified as the cells involved in controlling immunoglobulin E (IgE) production because of their ability to produce IL-4 and IL-13 and influence function of eosinophils through the actions of IL-5 (Romagnani 2004). In atopic individuals, allergen-specific-Th2 cytokines, particularly IL-4, IL-5, IL-9, and IL-13, orchestrate and amplify the $CD4^+$ Th2 response. In addition, Th2 and Th1 chemokines, including CCL11 and CXCL1, trigger the extravasation of and accumulation of eosinophils and neutrophils to perpetuate the allergic inflammation of the airways (Romagnani 2000, Zimmermann, Hershey et al. 2003). Furthermore, pro-inflammatory Th1 cytokines, such as IL-1β, IL-1α and IL-6, exacerbate lung inflammation (Jacquet 2011, Jacquet 2011, Willart, Deswarte et al. 2012, Sundaram, Mitra et al. 2015). Selected cytokines (TGF-β, IL-17 and IL-10) are involved in tissue remodeling (Bergeron, Tulic et al. 2010). Therefore, we examined levels of Th1, Th2 and Th17cytokines in WT and *caspase-11*$^{-/-}$ sera and BAL fluid samples. In the sera of HDM-treated mice, the levels of KC, TNFα and INFγ were respectively reduced, higher and similar to WT (Fig. 6A, C & E). However, the levels of these cytokines were reduced in the BAL of *caspase-11*$^{-/-}$ on day 35 (Fig. 6B, D & F). Furthermore, the level of IL-33 was not significantly different in the serum; yet, it was reduced in the BAL fluid of *caspase-11*$^{-/-}$ (Fig. 6G & H). No difference in the levels of serum IL-4, IL-5 and IL-10 (Fig. I, K & O) was observed between WT and *caspase11*$^{-/-}$ mice, while the levels of these cytokines were reduced in the BAL of *caspase11*$^{-/-}$ mice (Fig. 6J, L & P). The level of IL-17A was significantly higher in the serum but lower in the BAL fluid derived from *caspase-11*$^{-/-}$ (Fig. 6M & N). Importantly, we have tried to measure the levels of IL-13, IL-1α and IL-1β by the multiplex. However, these cytokines were below the detection level of the multiplex. Therefore, our data show that *caspase-11*$^{-/-}$ mice exhibit a reduction in the level of Th1, Th2 and Th17 cytokines in BAL fluid.

Discussion

HDM is one of the most common aeroallergens, inducing sensitization in approximately 85% of patients with asthma (Gregory and Lloyd 2011), and playing a pivotal role in initiating and perpetuating lung inflammation in patients with asthma (Maunsell, Wraith et al. 1968, Roche, Chinet et al. 1997, Hatzivlassiou, Grainge et al. 2010). Clinically, there is a strong correlation between the level of HDM exposure and sensitization, which is a strong predictor for asthma (Birrell, Van Oosterhout et al. 2010, De Alba, Raemdonck et al. 2010). The activation of the inflammasome in the lung can be triggered by stimuli such as HDM in the asthmatic airway (Madouri, Guillou et al. 2015). However, the role of the noncanonical inflammasome in asthma has not been elucidated. Herein, we used *caspase-11*$^{-/-}$ mice and macrophages from these mice to dissect whether caspase-11 plays a regulatory role in HDM induced allergic inflammation.

The study of macrophages in allergic asthma has focused on the role of alternatively activated AMs mediated by exposure to IL-4/IL-13. This macrophage subset positively correlated with the severity of airway inflammation in many studies (Draijer and Peters-Golden 2017). Adoptive transfer of *in vitro* differentiated IL-4/IL-13-stimulated macrophages into the lungs of allergic mice showed that these

macrophages actively contribute to the exacerbation of the disease and are not just bystanders (Melgert, Oriss et al. 2010). These findings were later confirmed by a few other studies (Moreira, Cavassani et al. 2010, Kim, Park et al. 2011, Chung, Lee et al. 2016), showing that alternatively activated macrophage enhance the allergic inflammatory responses in lung tissue. Our data show that *caspase-11*$^{-/-}$ macrophages treated with IL-4 expresse more markers of M2 cells than WT. Ym1 is overexpressed in asthma and aggravates lung injury (Chupp, Lee et al. 2007, Moreira and Hogaboam 2011). Even though macrophages express a stronger M2 phenotype, *caspase-11*$^{-/-}$ macrophages exhibit a significant reduction in expression of co-stimulatory molecules CD86, CD40 and MHCII 24 h post-treatment. Co-stimulation by macrophages is required to enhance the release of Th2 cytokines associated with asthma and B cells that will switch to IgE production. Our *in vivo* data show reduced IL-4 and other Th2 cytokines in the BALF of *caspase-11*$^{-/-}$ mice. Therefore, we speculate that reduced Th2 cytokines in *caspase-11*$^{-/-}$ lungs is associated with reduced M2 phenotypes and asthma features.

In our model, *caspase-11*$^{-/-}$ and WT mice exhibit similar drop in body temperature which is consistent with similar IgE level and similar lung functions. However, BAL fluids from *caspase-11*$^{-/-}$ mice show a significant increase in eosinophils despite lower IL-5 level. It has been demonstrated that eotaxin (an important eosinophil-specific chemokine that is associated with the recruitment of eosinophils into sites of inflammation), can promote tissue eosinophilia independent of IL-5 (Mould, Matthaei et al. 1997). We have tested the expression of CCL11 in lungs derived from *caspase-11*$^{-/-}$ mice challenged with HDM. Expression of CCL11 is higher in *caspase-11*$^{-/-}$ compared to WT lungs (Supplementary Fig. 1I). This finding could explain the increase in eosinophils in *caspase-11*$^{-/-}$ derived BAL fluid while IL-5 is reduced.

Additionally, *caspase-11*$^{-/-}$ mice exhibit reduced airway inflammation and reduced cellular infiltration including neutrophils, macrophages and lymphocytes in their lungs. The reduced infiltration of cells could be due to an inherent defect in their ability to migrate to sites of inflammation due to defects in the cytoskeleton (Li, Brieher et al. 2007, Caution, Young et al. 2019). We have shown that *capase-11*$^{-/-}$ neutrophils are defective in migration toward chemokines such as KC *in vivo* (Caution, Young et al. 2019). Furthermore, caspase-11 promotes neutrophil directional trafficking in an acute model of gout (Caution, Young et al. 2019). Others have shown that splenocytes and macrophages-derived from *caspase-11*$^{-/-}$ mice are defective in migration toward different chemokines *in vitro* and *in vivo* (Li, Brieher et al. 2007, Caution, Gavrilin et al. 2015). It was found that *caspase-11*$^{-/-}$ T cells migrate less efficiently into lymphoid tissues (Bergsbaken and Bevan 2015). Modulation of actin polymerization by caspase-11 could regulate additional aspects of T cell biology, including T cell receptor (TCR) signaling (Ritter, Angus et al. 2013) and hence affect their ability to migrate.

Further, BAL fluids of *caspase-11*$^{-/-}$ mice show increased total IgA. One other possibility for the reduced inflammation in *caspase-11*$^{-/-}$ mice could be associated with the increased level of total IgA in these mice. In addition to inhibition of bacterial colonization and neutralizing viruses at mucosal surfaces, (Williams and Gibbons 1972, Boyaka 2017), sIgA plays a protective role at mucosal surfaces by mediating tolerance and preventing hyper-inflammation toward allergens that can induce allergic

inflammation, such as asthma (Balzar, Strand et al. 2006, Gloudemans, Lambrecht et al. 2013). Therefore, it is plausible that the reduced lung inflammation in *caspase-11*$^{-/-}$ is associated with the increased level of IgA, which could potentially limit binding of HDM to host cells in our experimental model.

The reduced inflammation in *caspase-11*$^{-/-}$ lungs was evident by an overall reduction in KC, Th1 (TNFα and INFγ), Th2 (IL-33, IL-4, IL-5 and IL-10) and Th17 (IL-17A) levels in BAL fluid. *Caspase-11*$^{-/-}$ mice also show reduced level of IL-17A, which is secreted by a distinct subset of $CD4^+$ T helper cells and innate lymphoid cells (Yu, Kim et al. 2014). By secreting IL-17, Th17 cells orchestrate the recruitment of neutrophil granulocytes in the lungs and their activation directly through CXCL8 production (Pelletier, Maggi et al. 2010). Several reports have linked IL-17A production with asthma severity (Barczyk, Pierzchala et al. 2003, Sun, Zhou et al. 2005). Therefore, it is possible that the reduced number of neutrophils we found in the *caspase-11*$^{-/-}$ mice is due to lower IL-17A release.

The level of Th2 cytokines is reduced in the BAL fluid of *caspase 11*$^{-/-}$ mice. IL-33 is of particular importance since it is a chromatin-associated nuclear cytokine from the IL-1 family (Schmitz, Owyang et al. 2005). IL-33 is involved in polarization of T cells towards a Th2 phenotype and must be released extracellularly in order to bind to the ST2 receptor. Full length (FL) IL-33 is released passively during cell necrosis or when tissues are damaged, and functions as an alarmin (Cayrol and Girard 2009, Haraldsen, Balogh et al. 2009, Luthi, Cullen et al. 2009, Lefrancais, Roga et al. 2012, Cayrol and Girard 2014). While proteolysis of IL-33 by caspase-1 suppresses IL-33 bioactivity (Cayrol and Girard 2009, Luthi, Cullen et al. 2009, Talabot-Ayer, Lamacchia et al. 2009), inflammatory proteases including cathepsin G and neutrophil elastase also cleave IL-33 full length (FL) into a shorter mature form with higher activity than IL-33FL (Lefrancais, Roga et al. 2012, Morita, Nakae et al. 2017).

It has been shown that *caspase-1*$^{-/-}$*/caspase 11*$^{-/-}$ knockout mice exposed to HDM exhibit enhanced lung inflammation associated with a marked eosinophil recruitment, increased expression of IL-4, IL-5, IL-13, as well as full-length and cleaved bioactive IL-33 (Madouri, Guillou et al. 2015). In our *caspase-11*$^{-/-}$ mouse model, we detected less IL-33 in their BAL fluid and less Th2 cytokines associated with reduced inflammation. Given the role of IL-33 in enhancing Th2 cytokines production, we have seen reduced IL-5, IL-4 and IL-10. The reduction of Th2 cytokines could be a consequence of the reduced IL-33 in the BAL fluid.

On the inflammasome side, it is possible that caspase-1 is activated *in vivo* in the absence of caspase-11. We have seen a reduction in IL-1β level in *caspase-11*$^{-/-}$ macrophages treated with HDM *in vitro*. However, IL-1β level in WT and *caspase-11*$^{-/-}$ BAL fluid derived from mice challenged with HDM on day 35 were below the detection of the multiplex. Being a pro-inflammatory cytokine, it is possible that IL-1β level is more important at earlier time points in our model.

Even though we do not have a direct mechanism by which caspase-11 regulates airway inflammation in response to HDM, we speculate that lungs of *caspase-11*$^{-/-}$ mice exhibit global reduction in inflammation

seen by reduced chemokines, Th1, Th2 and Th17 cells. This reduction could impact cells recruitment into the lungs of these mice in response to HDM. Intriguingly, a new paper was published by Zaslona et al. on the role of *caspase-11*$^{-/-}$ in response to ova and alum. The authors demonstrated that *caspase-11*$^{-/-}$ mice show reduction in Th1, Th2 and Th17 cytokines in response to ova and alum (Zaslona, Flis et al. 2020). Even though we used a different allergen, exposure time and routes of administration, the Zaslona findings are in line with our data. Macrophages and dendritic cells experience the allergen early on and present processed fragments to naïve T cells. Following activation, T cells release cytokines that polarize immune cells and skew the environment toward asthma. It is possible that macrophages and dendritic cells show reduced ability to present the allergen to naïve T cells *in vivo*, which results in an overall reduction of cytokines and chemokines involved in cells polarization as well as the disease progression.

In conclusion, caspase-11 regulates lung inflammation in response to HDM during the priming and the effector phases of asthma. Our study offers several intriguing scenarios for the diverse functions of caspase-11. Expression of caspase-11 in innate immune cells following HDM exposure maybe required for the antigen presentation by macrophages and dendritic cells to naïve T cells. Subsequently, insufficient antigen presentation leads to reduction in cytokines and chemokines released in the BAL fluids. We have shown that caspase-11 regulates autophagy, which is an essential process for antigen presentation by immune cells{Krause, 2018 #733}. Our published work and that of others also support the notion for inherent defect in migration of *caspase-11*$^{-/-}$ cells due to defect in the actin cytoskeleton (Li, Brieher et al. 2007, Caution, Young et al. 2019). Further studies will explore these possibilities.

Materials And Methods

Mice

C57BL/6 WT mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). The *caspase-11*$^{-/-}$ mice were generously provided by Dr. Junying Yuan. (Harvard Medical School, Boston, MA, USA) (Wang, Miura et al. 1998). All mice are in the C57BL/6 background. All mice were housed in a pathogen-free facility and experiments were conducted with approval from the Animal Care and Use Committee at The Ohio State University (Columbus, OH, USA) in accordance with NIH and OSU IACUC guidelines. Mice were maintained in a temperature-controlled (23□C) facility with a strict 12-h light/ dark cycle and were given free access to food and water.

Cell culture and treatment of BMDMs with HDM and inflammatory stimuli

BMDMs from WT or *caspase-11*$^{-/-}$ were derived from the femurs of five to eight-week-old mice as previously described (Abu Khweek, Fernandez Davila et al. 2013, Khweek, Caution et al. 2013), and grown in IMDM (Iscove's modified Dulbecco's medium) supplemented with L929 fibroblast cell line (ATCC)-cultured supernatant and 10% heat inactivated fetal bovine serum (HIFBS) (Gibco). Cultivated macrophages were washed twice with PBS and incubated in IMDM media during treatments. Caspase-11 induction was measured by treating WT macrophages with various inflammatory mediators including IL-

1α (R&D Systems, 400-ML-005). (20ng/mL), IL-1β (20ng/mL) (R&D Systems, 401-ML-005), LPS (100ng/mL) (tlrl-eklps) for 4 h. WT macrophages were stimulated with IL-4 (5ng/mL) or IL-13 (20ng/mL) (PeproTech, 214-14, 210-13) for 1,4,6 and 24 h in IMDM media. In some instances, macrophages were pre-treated with IL-4 overnight, and then macrophages were un-treated or treated with HDM, HDM+ IL-4 for 4 or 24 h (Stallergenes Greer, XPB82D3A2.5). The vial was suspended with phosphate buffer saline to obtain 10mg/ml stock, then 10µl of the stock was used for each mouse which included ~7.85ng of endotoxin.

Induction of HDM-mediated allergic airway inflammation

To induce HDM-driven airway inflammation, mice were injected with HDM (100µg) adsorbed with alum (Imject^{TM} Alum, Thermo Scientific 77161) intraperitoneally on day 0 and day 7. Isoflurane anesthetized mice were challenged with (25µg) HDM intranasally for 15 challenges from day 14 until day 35. Twenty-four hours after the last challenge, mice were sacrificed for bronchoalveolar lavage (BAL), bronchial hyperresponsiveness, cytokines and chemokines concentration, histology and quantitative real time PCR.

Assessment of surface body temperature

Body temperature was assessed by measuring surface body temperature with the aid of infrared thermometers [Heat spy infrared thermal imaging camera (Wahl, Culver City, CA, USA)]. Mice were incubated for 10 minutes at room temperature, and then their body temperatures were measured. Subsequently, mice were challenged with HDM and incubated for 30 minutes, and their body temperatures were measured again. Then, the body temperature after the challenge was subtracted from that before the challenge for each mouse. This is shown as drop in body temperature as indicated in the figures.

Determination of bronchial hyper-responsiveness

Lung function (respiratory system resistance) was assessed using the forced-oscillation technique (Irvin and Bates 2003). Briefly, mice were sedated via intra-peritoneal injection of diazepam/Valium (70µl). Ten minutes after, ketamine was administered via intra-peritoneal injection according to mice weight. For instance, mice weighed above or below 25g were given 90µl or 70µl of ketamine, respectively. Mice were paralyzed by intra-muscular injections of rocuronium and intubated with an 18-gauge catheter. Anesthetized mice were mechanically ventilated on a flexiVent computer-controlled piston ventilator (SCIreq, Montreal, PC, Canada) with 8 ml/kg tidal volume at a frequency of 150 breaths/min against 2–3 cmH2O positive end-expiratory pressure (Irvin and Bates 2003). Airway responsiveness to bronchoconstrictors was measured following exposure to increasing concentrations of methacholine (0.1–50 mg/ml) (Davis, Xu et al. 2007).

Bronchoalveolar lavage (BAL) and flow cytometry of lung mononuclear cells

Bronchoalveolar lavage was performed by washing the lungs once with 0.8 ml of saline solution at room temperature. After centrifugation at 1500RPM for 5 min at 4□C, the supernatant (cell-free BAL fluid) was stored at -20□C for cytokine analysis. To determine the percentages of inflammatory cells infiltration in BAL fluid, cells were suspended in FACS buffer and either unstained or single/ multiple stained with the following antibodies: anti CD3 (T-cells), anti CD8 (cytotoxic T cells), anti CD19 (B cells), anti C-kit (mast cells), anti SiglecF (eosinophils), anti CD49b (NK-cells) and anti CD11c/F480 (alveolar macrophages). Stained cells were analyzed with an Attune NxT flow cytometer (Thermo Fisher Scientific, Waltham, MA).

Assessment of HDM-specific IgG1 and IgA Ab responses and total IgA Levels

Total and HDM-specific Ab responses were measured in sera and BALF by enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates were coated with HDM (25 µg/ml). For detection of HDM-specific IgG1 and IgA Abs, serial dilutions of serum or BALFs were added to the plates and the binding antibodies were detected with HRP-conjugated antisera (Southern Biotech Associates Inc., Birmingham, AL, USA). Biotin-conjugated rat anti-mouse IgG1 monoclonal Abs and HRP-conjugated streptavidin (BD Bioscience, San Jose, CA, ISA) were used to measure IgG1. The Ab titers were determined as the last dilutions of samples that with an absorbance of >0.1 above that of control samples. Total IgA levels were determined by ELISA using extrapolation against IgA standards (Duverger, Jackson et al. 2006).

Total IgE quantification

Total IgE Ab levels were determined by a mouse IgE ELISA kit (Thermofischer scientific). Sample dilution and IgE detection were done according to instructions from the manufacturer.

Real-Time PCR

Tissues were collected; snap frozen, and reduced to powder before adding TRIzol (Invitrogen, Carlsbad, CA, USA). RNA was isolated and cDNA was synthesized by using Superscript III (Invitrogen) and real-time PCR was performed as previously described (Gavrilin, Bouakl et al. 2006, Abu Khweek, Kanneganti et al. 2016) with the aid of specific primers. The gene of interest Ct was normalized to β-actin Ct and expressed as relative copy number (RCN) of mRNA expression (RCN= $2^{-\Delta Ct}$).

Lung Histology

The right lungs from each group (n=5 per group) were fixated with 4 % buffered formalin solution. Sections were stained with hematoxylin and eosin (H&E) for quantifying inflammation and PAS (Periodic Acid Schiff, Sigma-Aldrich) for quantifying mucus producing goblet cells. Sectioning, staining and scoring were performed at the Ohio state veterinary school. Several parameters were evaluated including perivesicular, peribronchiolar and parenchymal inflammation, inflammatory cells in blood vessel walls, perivascular and peribronchiolar edema, alveolar macrophages distant to affected airways/blood vessels, predominance of neutrophils and goblet cell mucus in bronchioles. Scoring scale was (0-5) where, 0, 1,2,3,4 and 5 represents no signs, mild (<25%), moderate (26-50%), marked (51-75%), Severe (> 75%), and

maximum inflammation. The semi-quantitative scoring rubric used to produce our data was developed and modified by board-certified comparative veterinary pathologist, Dr. Kara N. Corps, DVM, PhD, DACVP, at the Comparative Pathology and Mouse Phenotyping Shared Resource of The Ohio State University. Dr. Corps produced the scoring system based on the requirements of our project and employing standard semi-quantitative methods. The included manuscripts are examples from our field of similar methods and were referenced by Dr. Corps in developing our standardized method, which was used for all semi-quantitative histopathology scoring data reported in this manuscript (Clarke, Davis et al. 2014, Anas, Yang et al. 2017).

Western blotting

Protein extraction from macrophages was performed using TRIzol™ reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Briefly, after phase separation using chloroform, 100% ethanol was added to the interphase/phenol-chloroform layer to precipitate genomic DNA. Subsequently, the phenol-ethanol supernatant was mixed with isopropanol in order to precipitate out proteins. Isolated protein was then denatured in a urea-based lysis buffer. The Bradford method was used to determine protein concentrations. Equal amounts of protein were separated by 12% SDS-polyacrylamide gel (Biorad, 161-0158) and transferred to a polyvinylidene fluoride (PVDF) membrane (Biorad, 162-0177). Membranes were immunoblotted with antibodies that recognize caspase-11 (Sigma Aldrich, C1354), GAPDH (Cell Signaling Technology, 2118), Phospho-Stat6 (Tyr641) (D8S9Y) (Cell Signaling Technology, 56554), total Stat6 D3H4 (Cell Signaling Technology, 5394). Corresponding secondary antibodies conjugated with horseradish peroxidase (Cell Signaling Technology, 7074; Santa Cruz Biotechnology, sc-2006) and in combination with enhanced chemiluminescence reagent (Amersham, RPN2209,) were used to visualize protein bands

Cytokine measurements

Cytokines including IL-1β, KC, TNFα, IL-4, IL-5, IL-13, IL-10, INFγ and IL-33, were measured in serum and BAL fluid samples using multiplex ELISA according to the manufacturer recommendations at the center for Clinical and Translational Science (CCTS) at Ohio State University. The level of IL-1β, IL-6, IL-12 and KC in the supernatant of treated macrophages was determined by specific sandwich ELISA following the manufacturer's protocol (R&D) system Inc.

Statistical analysis

Figures display the mean and standard deviation (SD) as indicated in the figure legends. Comparisons between groups were conducted by multiple t-test, two-way ANOVA or linear mixed effects models were used for analysis to take account of the correlation among observations from the same animal within the same cage. Holm's procedure was used to adjust for multiple comparisons or multiple outcomes to control for type I error at 0.05. Mice were age and sex matched, however no difference was seen in the gender. We did two independent in vivo experiments with seven and five mice per group, respectively which lead to similar results. The numbers of animal/group are indicated for each statistical analysis and

this is indicated in the figure legends. Statistically significant differences were defined as *P < 0.05, **P < 0.01, and ***P < 0.001.

Declarations

Ethics Statement

The animal study was reviewed and approved by OSU IACUC.

Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Availability of data and materials

All the obtained data are available in the laboratory of Dr. Amal Amer and Dr. Prosper N Boyaka.

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Author Contributions

AAK designed, performed analyzed, interpreted data and wrote, edited, and reviewed the manuscript. MRJ assisted in performing *in vivo* experiments. EK, ZA, KK, KD, SE, KH, AB and MAG contributed by performing experiments and editing the manuscript. PNB helped in designing, conceptualizing experiments, interpreting data, editing and reviewing the manuscript. XZ performed statistical analysis. AOA assisted in the experimental design and implementation, interpretation of data, as well as editing the manuscript.

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References

Abu Khweek, A., N. S. Fernandez Davila, K. Caution, A. Akhter, B. A. Abdulrahman, M. Tazi, H. Hassan, L. A. Novotny, L. O. Bakaletz and A. O. Amer (2013). "Biofilm-derived Legionella pneumophila evades the innate immune response in macrophages." Front Cell Infect Microbiol **3**: 18.

Abu Khweek, A., A. Kanneganti, D. D. Guttridge and A. O. Amer (2016). "The Sphingosine-1-Phosphate Lyase (LegS2) Contributes to the Restriction of Legionella pneumophila in Murine Macrophages." PLoS One **11**(1): e0146410.

Anas, A. A., J. Yang, J. Daan de Boer, J. J. Roelofs, B. Hou, A. F. de Vos and T. van der Poll (2017). "General, but not myeloid or type II lung epithelial cell, myeloid differentiation factor 88 deficiency abrogates house dust mite induced allergic lung inflammation." Clin Exp Immunol **187**(2): 204-212.

Arlian, L. G. and T. A. Platts-Mills (2001). "The biology of dust mites and the remediation of mite allergens in allergic disease." J Allergy Clin Immunol **107**(3 Suppl): S406-413.

Asokananthan, N., P. T. Graham, D. J. Stewart, A. J. Bakker, K. A. Eidne, P. J. Thompson and G. A. Stewart (2002). "House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1." J Immunol **169**(8): 4572-4578.

Awad, F., E. Assrawi, C. Jumeau, S. Georgin-Lavialle, L. Cobret, P. Duquesnoy, W. Piterboth, L. Thomas, K. Stankovic-Stojanovic, C. Louvrier, I. Giurgea, G. Grateau, S. Amselem and S. A. Karabina (2017). "Impact of human monocyte and macrophage polarization on NLR expression and NLRP3 inflammasome activation." PLoS One **12**(4): e0175336.

Balzar, S., M. Strand, T. Nakano and S. E. Wenzel (2006). "Subtle immunodeficiency in severe asthma: IgA and IgG2 correlate with lung function and symptoms." Int Arch Allergy Immunol **140**(2): 96-102.

Barczyk, A., W. Pierzchala and E. Sozanska (2003). "Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine." Respir Med **97**(6): 726-733.

Bauernfeind, F. G., G. Horvath, A. Stutz, E. S. Alnemri, K. MacDonald, D. Speert, T. Fernandes-Alnemri, J. Wu, B. G. Monks, K. A. Fitzgerald, V. Hornung and E. Latz (2009). "Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression." J Immunol **183**(2): 787-791.

Bergeron, C., M. K. Tulic and Q. Hamid (2010). "Airway remodelling in asthma: from benchside to clinical practice." Can Respir J **17**(4): e85-93.

Bergsbaken, T. and M. J. Bevan (2015). "Cutting Edge: Caspase-11 Limits the Response of CD8+ T Cells to Low-Abundance and Low-Affinity Antigens." J Immunol **195**(1): 41-45.

Bhatia, S., M. Fei, M. Yarlagadda, Z. Qi, S. Akira, S. Saijo, Y. Iwakura, N. van Rooijen, G. A. Gibson, C. M. St Croix, A. Ray and P. Ray (2011). "Rapid host defense against Aspergillus fumigatus involves alveolar macrophages with a predominance of alternatively activated phenotype." PLoS One **6**(1): e15943.

Birrell, M. A., A. J. Van Oosterhout and M. G. Belvisi (2010). "Do the current house dust mite-driven models really mimic allergic asthma?" Eur Respir J **36**(5): 1220-1221.

Bordas-Le Floch, V., M. Le Mignon, L. Bussieres, K. Jain, A. Martelet, V. Baron-Bodo, E. Nony, L. Mascarell and P. Moingeon (2017). "A combined transcriptome and proteome analysis extends the allergome of house dust mite Dermatophagoides species." PLoS One **12**(10): e0185830.

Boyaka, P. N. (2017). "Inducing Mucosal IgA: A Challenge for Vaccine Adjuvants and Delivery Systems." J Immunol **199**(1): 9-16.

Broz, P. and V. M. Dixit (2016). "Inflammasomes: mechanism of assembly, regulation and signalling." Nat Rev Immunol **16**(7): 407-420.

Broz, P., T. Ruby, K. Belhocine, D. M. Bouley, N. Kayagaki, V. M. Dixit and D. M. Monack (2012). "Caspase-11 increases susceptibility to Salmonella infection in the absence of caspase-1." Nature **490**(7419): 288-291.

Calderon, M. A., A. Linneberg, J. Kleine-Tebbe, F. De Blay, D. Hernandez Fernandez de Rojas, J. C. Virchow and P. Demoly (2015). "Respiratory allergy caused by house dust mites: What do we really know?" J Allergy Clin Immunol **136**(1): 38-48.

Caution, K., M. A. Gavrilin, M. Tazi, A. Kanneganti, D. Layman, S. Hoque, K. Krause and A. O. Amer (2015). "Caspase-11 and caspase-1 differentially modulate actin polymerization via RhoA and Slingshot proteins to promote bacterial clearance." Sci Rep **5**: 18479.

Caution, K., N. Young, F. Robledo-Avila, K. Krause, A. Abu Khweek, K. Hamilton, A. Badr, A. Vaidya, K. Daily, H. Gosu, M. N. K. Anne, M. Eltobgy, D. Dakhlallah, S. Argwal, S. Estfanous, X. Zhang, S. Partida-Sanchez, M. A. Gavrilin, W. N. Jarjour and A. O. Amer (2019). "Caspase-11 Mediates Neutrophil Chemotaxis and Extracellular Trap Formation During Acute Gouty Arthritis Through Alteration of Cofilin Phosphorylation." Front Immunol **10**: 2519.

Cayrol, C. and J. P. Girard (2009). "The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1." Proc Natl Acad Sci U S A **106**(22): 9021-9026.

Cayrol, C. and J. P. Girard (2014). "IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy." Curr Opin Immunol **31**: 31-37.

Chung, S., T. J. Lee, B. F. Reader, J. Y. Kim, Y. G. Lee, G. Y. Park, M. Karpurapu, M. N. Ballinger, F. Qian, L. Rusu, H. Y. Chung, T. G. Unterman, C. M. Croce and J. W. Christman (2016). "FoxO1 regulates allergic asthmatic inflammation through regulating polarization of the macrophage inflammatory phenotype." Oncotarget **7**(14): 17532-17546.

Chupp, G. L., C. G. Lee, N. Jarjour, Y. M. Shim, C. T. Holm, S. He, J. D. Dziura, J. Reed, A. J. Coyle, P. Kiener, M. Cullen, M. Grandsaigne, M. C. Dombret, M. Aubier, M. Pretolani and J. A. Elias (2007). "A chitinase-like protein in the lung and circulation of patients with severe asthma." N Engl J Med **357**(20): 2016-2027.

Clarke, D. L., N. H. Davis, C. L. Campion, M. L. Foster, S. C. Heasman, A. R. Lewis, I. K. Anderson, D. J. Corkill, M. A. Sleeman, R. D. May and M. J. Robinson (2014). "Dectin-2 sensing of house dust mite is critical for the initiation of airway inflammation." Mucosal Immunol **7**(3): 558-567.

Davis, I. C., A. Xu, Z. Gao, J. M. Hickman-Davis, P. Factor, W. M. Sullender and S. Matalon (2007). "Respiratory syncytial virus induces insensitivity to beta-adrenergic agonists in mouse lung epithelium in vivo." Am J Physiol Lung Cell Mol Physiol **293**(2): L281-289.

De Alba, J., K. Raemdonck, A. Dekkak, M. Collins, S. Wong, A. T. Nials, R. G. Knowles, M. G. Belvisi and M. A. Birrell (2010). "House dust mite induces direct airway inflammation in vivo: implications for future disease therapy?" Eur Respir J **35**(6): 1377-1387.

Draijer, C. and M. Peters-Golden (2017). "Alveolar Macrophages in Allergic Asthma: the Forgotten Cell Awakes." Curr Allergy Asthma Rep **17**(2): 12.

Dullaers, M., R. De Bruyne, F. Ramadani, H. J. Gould, P. Gevaert and B. N. Lambrecht (2012). "The who, where, and when of IgE in allergic airway disease." J Allergy Clin Immunol **129**(3): 635-645.

Duverger, A., R. J. Jackson, F. W. van Ginkel, R. Fischer, A. Tafaro, S. H. Leppla, K. Fujihashi, H. Kiyono, J. R. McGhee and P. N. Boyaka (2006). "Bacillus anthracis edema toxin acts as an adjuvant for mucosal immune responses to nasally administered vaccine antigens." J Immunol **176**(3): 1776-1783.

Faucheu, C., A. M. Blanchet, V. Collard-Dutilleul, J. L. Lalanne and A. Diu-Hercend (1996). "Identification of a cysteine protease closely related to interleukin-1 beta-converting enzyme." Eur J Biochem **236**(1): 207-213.

Faucheu, C., A. Diu, A. W. Chan, A. M. Blanchet, C. Miossec, F. Herve, V. Collard-Dutilleul, Y. Gu, R. A. Aldape, J. A. Lippke and et al. (1995). "A novel human protease similar to the interleukin-1 beta converting enzyme induces apoptosis in transfected cells." EMBO J **14**(9): 1914-1922.

Gavrilin, M. A., I. J. Bouakl, N. L. Knatz, M. D. Duncan, M. W. Hall, J. S. Gunn and M. D. Wewers (2006). "Internalization and phagosome escape required for Francisella to induce human monocyte IL-1beta processing and release." Proc Natl Acad Sci U S A **103**(1): 141-146.

Gloudemans, A. K., B. N. Lambrecht and H. H. Smits (2013). "Potential of immunoglobulin A to prevent allergic asthma." Clin Dev Immunol **2013**: 542091.

Gordon, S. (2003). "Alternative activation of macrophages." Nat Rev Immunol **3**(1): 23-35.

Greenfield, E. A., K. A. Nguyen and V. K. Kuchroo (1998). "CD28/B7 costimulation: a review." Crit Rev Immunol **18**(5): 389-418.

Gregory, L. G. and C. M. Lloyd (2011). "Orchestrating house dust mite-associated allergy in the lung." Trends Immunol **32**(9): 402-411.

Grewal, I. S. and R. A. Flavell (1998). "CD40 and CD154 in cell-mediated immunity." Annu Rev Immunol **16**: 111-135.

Gurung, P., R. K. Malireddi, P. K. Anand, D. Demon, L. Vande Walle, Z. Liu, P. Vogel, M. Lamkanfi and T. D. Kanneganti (2012). "Toll or interleukin-1 receptor (TIR) domain-containing adaptor inducing interferon-beta (TRIF)-mediated caspase-11 protease production integrates Toll-like receptor 4 (TLR4) protein- and Nlrp3 inflammasome-mediated host defense against enteropathogens." J Biol Chem **287**(41): 34474-34483.

Hagar, J. A., D. A. Powell, Y. Aachoui, R. K. Ernst and E. A. Miao (2013). "Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock." Science **341**(6151): 1250-1253.

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**(4): 410-416.

Haraldsen, G., J. Balogh, J. Pollheimer, J. Sponheim and A. M. Kuchler (2009). "Interleukin-33 - cytokine of dual function or novel alarmin?" Trends Immunol **30**(5): 227-233.

Hatzivlassiou, M., C. Grainge, V. Kehagia, L. Lau and P. H. Howarth (2010). "The allergen specificity of the late asthmatic reaction." Allergy **65**(3): 355-358.

Hong, J. Y., Y. Chung, J. Steenrod, Q. Chen, J. Lei, A. T. Comstock, A. M. Goldsmith, J. K. Bentley, U. S. Sajjan and M. B. Hershenson (2014). "Macrophage activation state determines the response to rhinovirus infection in a mouse model of allergic asthma." Respir Res **15**: 63.

Irvin, C. G. and J. H. Bates (2003). "Measuring the lung function in the mouse: the challenge of size." Respir Res **4**: 4.

Jacquet, A. (2011). "The role of innate immunity activation in house dust mite allergy." Trends Mol Med **17**(10): 604-611.

Jacquet, A. (2011). "The role of the house dust mite-induced innate immunity in development of allergic response." Int Arch Allergy Immunol **155**(2): 95-105.

Janeway, C. A., Jr. and R. Medzhitov (2002). "Innate immune recognition." Annu Rev Immunol **20**: 197-216.

Kamens, J., M. Paskind, M. Hugunin, R. V. Talanian, H. Allen, D. Banach, N. Bump, M. Hackett, C. G. Johnston, P. Li and et al. (1995). "Identification and characterization of ICH-2, a novel member of the interleukin-1 beta-converting enzyme family of cysteine proteases." J Biol Chem **270**(25): 15250-15256.

Kato, T., T. Takai, T. Fujimura, H. Matsuoka, T. Ogawa, K. Murayama, A. Ishii, S. Ikeda, K. Okumura and H. Ogawa (2009). "Mite serine protease activates protease-activated receptor-2 and induces cytokine release in human keratinocytes." Allergy **64**(9): 1366-1374.

Kawai, T. and S. Akira (2010). "The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors." Nat Immunol **11**(5): 373-384.

Kay, A. B. (2001). "Allergy and allergic diseases. First of two parts." N Engl J Med **344**(1): 30-37.

Kayagaki, N., S. Warming, M. Lamkanfi, L. Vande Walle, S. Louie, J. Dong, K. Newton, Y. Qu, J. Liu, S. Heldens, J. Zhang, W. P. Lee, M. Roose-Girma and V. M. Dixit (2011). "Non-canonical inflammasome activation targets caspase-11." Nature **479**(7371): 117-121.

Kayagaki, N., M. T. Wong, I. B. Stowe, S. R. Ramani, L. C. Gonzalez, S. Akashi-Takamura, K. Miyake, J. Zhang, W. P. Lee, A. Muszynski, L. S. Forsberg, R. W. Carlson and V. M. Dixit (2013). "Noncanonical inflammasome activation by intracellular LPS independent of TLR4." Science **341**(6151): 1246-1249.

Khweek, A. A., K. Caution, A. Akhter, B. A. Abdulrahman, M. Tazi, H. Hassan, N. Majumdar, A. Doran, E. Guirado, L. S. Schlesinger, H. Shuman and A. O. Amer (2013). "A bacterial protein promotes the recognition of the Legionella pneumophila vacuole by autophagy." Eur J Immunol **43**(5): 1333-1344.

Kim, D. Y., B. S. Park, G. U. Hong, B. J. Lee, J. W. Park, S. Y. Kim and J. Y. Ro (2011). "Anti-inflammatory effects of the R2 peptide, an inhibitor of transglutaminase 2, in a mouse model of allergic asthma, induced by ovalbumin." Br J Pharmacol **162**(1): 210-225.

Kubo, M. (2017). "Innate and adaptive type 2 immunity in lung allergic inflammation." Immunol Rev **278**(1): 162-172.

Lamkanfi, M., W. Declercq, M. Kalai, X. Saelens and P. Vandenabeele (2002). "Alice in caspase land. A phylogenetic analysis of caspases from worm to man." Cell Death Differ **9**(4): 358-361.

Lamkanfi, M. and V. M. Dixit (2014). "Mechanisms and functions of inflammasomes." Cell **157**(5): 1013-1022.

Lefrancais, E., S. Roga, V. Gautier, A. Gonzalez-de-Peredo, B. Monsarrat, J. P. Girard and C. Cayrol (2012). "IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G." Proc Natl Acad Sci U S A **109**(5): 1673-1678.

Li, J., W. M. Brieher, M. L. Scimone, S. J. Kang, H. Zhu, H. Yin, U. H. von Andrian, T. Mitchison and J. Yuan (2007). "Caspase-11 regulates cell migration by promoting Aip1-Cofilin-mediated actin depolymerization." Nat Cell Biol **9**(3): 276-286.

Lin, X. Y., M. S. Choi and A. G. Porter (2000). "Expression analysis of the human caspase-1 subfamily reveals specific regulation of the CASP5 gene by lipopolysaccharide and interferon-gamma." J Biol Chem **275**(51): 39920-39926.

Luthi, A. U., S. P. Cullen, E. A. McNeela, P. J. Duriez, I. S. Afonina, C. Sheridan, G. Brumatti, R. C. Taylor, K. Kersse, P. Vandenabeele, E. C. Lavelle and S. J. Martin (2009). "Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases." Immunity **31**(1): 84-98.

Madouri, F., N. Guillou, L. Fauconnier, T. Marchiol, N. Rouxel, P. Chenuet, A. Ledru, L. Apetoh, F. Ghiringhelli, M. Chamaillard, S. G. Zheng, F. Trovero, V. F. Quesniaux, B. Ryffel and D. Togbe (2015). "Caspase-1 activation by NLRP3 inflammasome dampens IL-33-dependent house dust mite-induced allergic lung inflammation." J Mol Cell Biol **7**(4): 351-365.

Martinon, F. and J. Tschopp (2004). "Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases." Cell **117**(5): 561-574.

Maunsell, K., D. G. Wraith and A. M. Cunnington (1968). "Mites and house-dust allergy in bronchial asthma." Lancet **1**(7555): 1267-1270.

McAdam, A. J., A. N. Schweitzer and A. H. Sharpe (1998). "The role of B7 co-stimulation in activation and differentiation of CD4+ and CD8+ T cells." Immunol Rev **165**: 231-247.

Melgert, B. N., T. B. Oriss, Z. Qi, B. Dixon-McCarthy, M. Geerlings, M. N. Hylkema and A. Ray (2010). "Macrophages: regulators of sex differences in asthma?" Am J Respir Cell Mol Biol **42**(5): 595-603.

Moreira, A. P., K. A. Cavassani, R. Hullinger, R. S. Rosada, D. J. Fong, L. Murray, D. P. Hesson and C. M. Hogaboam (2010). "Serum amyloid P attenuates M2 macrophage activation and protects against fungal spore-induced allergic airway disease." J Allergy Clin Immunol **126**(4): 712-721.e717.

Moreira, A. P. and C. M. Hogaboam (2011). "Macrophages in allergic asthma: fine-tuning their pro- and anti-inflammatory actions for disease resolution." J Interferon Cytokine Res **31**(6): 485-491.

Morita, H., S. Nakae, H. Saito and K. Matsumoto (2017). "IL-33 in clinical practice: Size matters?" J Allergy Clin Immunol **140**(2): 381-383.

Mould, A. W., K. I. Matthaei, I. G. Young and P. S. Foster (1997). "Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice." J Clin Invest **99**(5): 1064-1071.

Munday, N. A., J. P. Vaillancourt, A. Ali, F. J. Casano, D. K. Miller, S. M. Molineaux, T. T. Yamin, V. L. Yu and D. W. Nicholson (1995). "Molecular cloning and pro-apoptotic activity of ICErelII and ICErelIII, members of the ICE/CED-3 family of cysteine proteases." J Biol Chem **270**(26): 15870-15876.

Na, H., M. Cho and Y. Chung (2016). "Regulation of Th2 Cell Immunity by Dendritic Cells." Immune Netw **16**(1): 1-12.

Pelletier, M., L. Maggi, A. Micheletti, E. Lazzeri, N. Tamassia, C. Costantini, L. Cosmi, C. Lunardi, F. Annunziato, S. Romagnani and M. A. Cassatella (2010). "Evidence for a cross-talk between human neutrophils and Th17 cells." Blood **115**(2): 335-343.

Platts-Mills, T. A. and M. D. Chapman (1987). "Dust mites: immunology, allergic disease, and environmental control." J Allergy Clin Immunol **80**(6): 755-775.

Rathinam, V. A., S. K. Vanaja, L. Waggoner, A. Sokolovska, C. Becker, L. M. Stuart, J. M. Leong and K. A. Fitzgerald (2012). "TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by gram-negative bacteria." Cell **150**(3): 606-619.

Ricci, M., O. Rossi, M. Bertoni and A. Matucci (1993). "The importance of Th2-like cells in the pathogenesis of airway allergic inflammation." Clin Exp Allergy **23**(5): 360-369.

Ritter, A. T., K. L. Angus and G. M. Griffiths (2013). "The role of the cytoskeleton at the immunological synapse." Immunol Rev **256**(1): 107-117.

Roche, N., T. C. Chinet and G. J. Huchon (1997). "Allergic and nonallergic interactions between house dust mite allergens and airway mucosa." Eur Respir J **10**(3): 719-726.

Romagnani, S. (2000). "The role of lymphocytes in allergic disease." J Allergy Clin Immunol **105**(3): 399-408.

Romagnani, S. (2004). "Immunologic influences on allergy and the TH1/TH2 balance." J Allergy Clin Immunol **113**(3): 395-400.

Sanchez-Borges, M., E. Fernandez-Caldas, W. R. Thomas, M. D. Chapman, B. W. Lee, L. Caraballo, N. Acevedo, F. T. Chew, I. J. Ansotegui, L. Behrooz, W. Phipatanakul, R. Gerth van Wijk, D. Pascal, N. Rosario, M. Ebisawa, M. Geller, S. Quirce, S. Vrtala, R. Valenta, M. Ollert, G. W. Canonica, M. A. Calderon, C. S. Barnes, A. Custovic, S. Benjaponpitak and A. Capriles-Hulett (2017). "International consensus (ICON) on: clinical consequences of mite hypersensitivity, a global problem." World Allergy Organ J **10**(1): 14.

Schauvliege, R., J. Vanrobaeys, P. Schotte and R. Beyaert (2002). "Caspase-11 gene expression in response to lipopolysaccharide and interferon-gamma requires nuclear factor-kappa B and signal

transducer and activator of transcription (STAT) 1." J Biol Chem **277**(44): 41624-41630.

Schmitz, J., A. Owyang, E. Oldham, Y. Song, E. Murphy, T. K. McClanahan, G. Zurawski, M. Moshrefi, J. Qin, X. Li, D. M. Gorman, J. F. Bazan and R. A. Kastelein (2005). "IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines." Immunity **23**(5): 479-490.

Sica, A. and A. Mantovani (2012). "Macrophage plasticity and polarization: in vivo veritas." J Clin Invest **122**(3): 787-795.

Sun, Y. C., Q. T. Zhou and W. Z. Yao (2005). "Sputum interleukin-17 is increased and associated with airway neutrophilia in patients with severe asthma." Chin Med J (Engl) **118**(11): 953-956.

Sundaram, K., S. Mitra, M. A. Gavrilin and M. D. Wewers (2015). "House Dust Mite Allergens and the Induction of Monocyte Interleukin 1beta Production That Triggers an IkappaBzeta-Dependent Granulocyte Macrophage Colony-Stimulating Factor Release from Human Lung Epithelial Cells." Am J Respir Cell Mol Biol **53**(3): 400-411.

Talabot-Ayer, D., C. Lamacchia, C. Gabay and G. Palmer (2009). "Interleukin-33 is biologically active independently of caspase-1 cleavage." J Biol Chem **284**(29): 19420-19426.

Thomas, W. R. (2012). "House dust allergy and immunotherapy." Hum Vaccin Immunother **8**(10): 1469-1478.

Thomas, W. R., B. J. Hales and W. A. Smith (2010). "House dust mite allergens in asthma and allergy." Trends Mol Med **16**(7): 321-328.

Wahn, U., S. Lau, R. Bergmann, M. Kulig, J. Forster, K. Bergmann, C. P. Bauer and I. Guggenmoos-Holzmann (1997). "Indoor allergen exposure is a risk factor for sensitization during the first three years of life." J Allergy Clin Immunol **99**(6 Pt 1): 763-769.

Wambre, E., E. A. James and W. W. Kwok (2012). "Characterization of CD4+ T cell subsets in allergy." Curr Opin Immunol **24**(6): 700-706.

Wang, S., M. Miura, Y. K. Jung, H. Zhu, E. Li and J. Yuan (1998). "Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE." Cell **92**(4): 501-509.

Willart, M. A., K. Deswarte, P. Pouliot, H. Braun, R. Beyaert, B. N. Lambrecht and H. Hammad (2012). "Interleukin-1alpha controls allergic sensitization to inhaled house dust mite via the epithelial release of GM-CSF and IL-33." J Exp Med **209**(8): 1505-1517.

Willart, M. A. and B. N. Lambrecht (2009). "The danger within: endogenous danger signals, atopy and asthma." Clin Exp Allergy **39**(1): 12-19.

Williams, R. C. and R. J. Gibbons (1972). "Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal." Science **177**(4050): 697-699.

Woodfolk, J. A. (2007). "T-cell responses to allergens." J Allergy Clin Immunol **119**(2): 280-294; quiz 295-286.

Yang, J., Y. Zhao and F. Shao (2015). "Non-canonical activation of inflammatory caspases by cytosolic LPS in innate immunity." Curr Opin Immunol **32**: 78-83.

Yu, S., H. Y. Kim, Y. J. Chang, R. H. DeKruyff and D. T. Umetsu (2014). "Innate lymphoid cells and asthma." J Allergy Clin Immunol **133**(4): 943-950; quiz 951.

Zaslona, Z., E. Flis, M. M. Wilk, R. G. Carroll, E. M. Palsson-McDermott, M. M. Hughes, C. Diskin, K. Banahan, D. G. Ryan, A. Hooftman, A. Misiak, J. Kearney, G. Lochnit, W. Bertrams, T. Greulich, B. Schmeck, O. J. McElvaney, K. H. G. Mills, E. C. Lavelle, M. Wygrecka, E. M. Creagh and L. A. J. O'Neill (2020). "Caspase-11 promotes allergic airway inflammation." Nat Commun **11**(1): 1055.

Zhong, B., X. Yang, Q. Sun, L. Liu, X. Lan, J. Tian, Q. He, W. Hou, H. Liu, C. Jiang, N. Gao and S. Lu (2014). "Pdcd4 modulates markers of macrophage alternative activation and airway remodeling in antigen-induced pulmonary inflammation." J Leukoc Biol **96**(6): 1065-1075.

Zimmermann, N., G. K. Hershey, P. S. Foster and M. E. Rothenberg (2003). "Chemokines in asthma: cooperative interaction between chemokines and IL-13." J Allergy Clin Immunol **111**(2): 227-242; quiz 243.

Figures

Fig.1

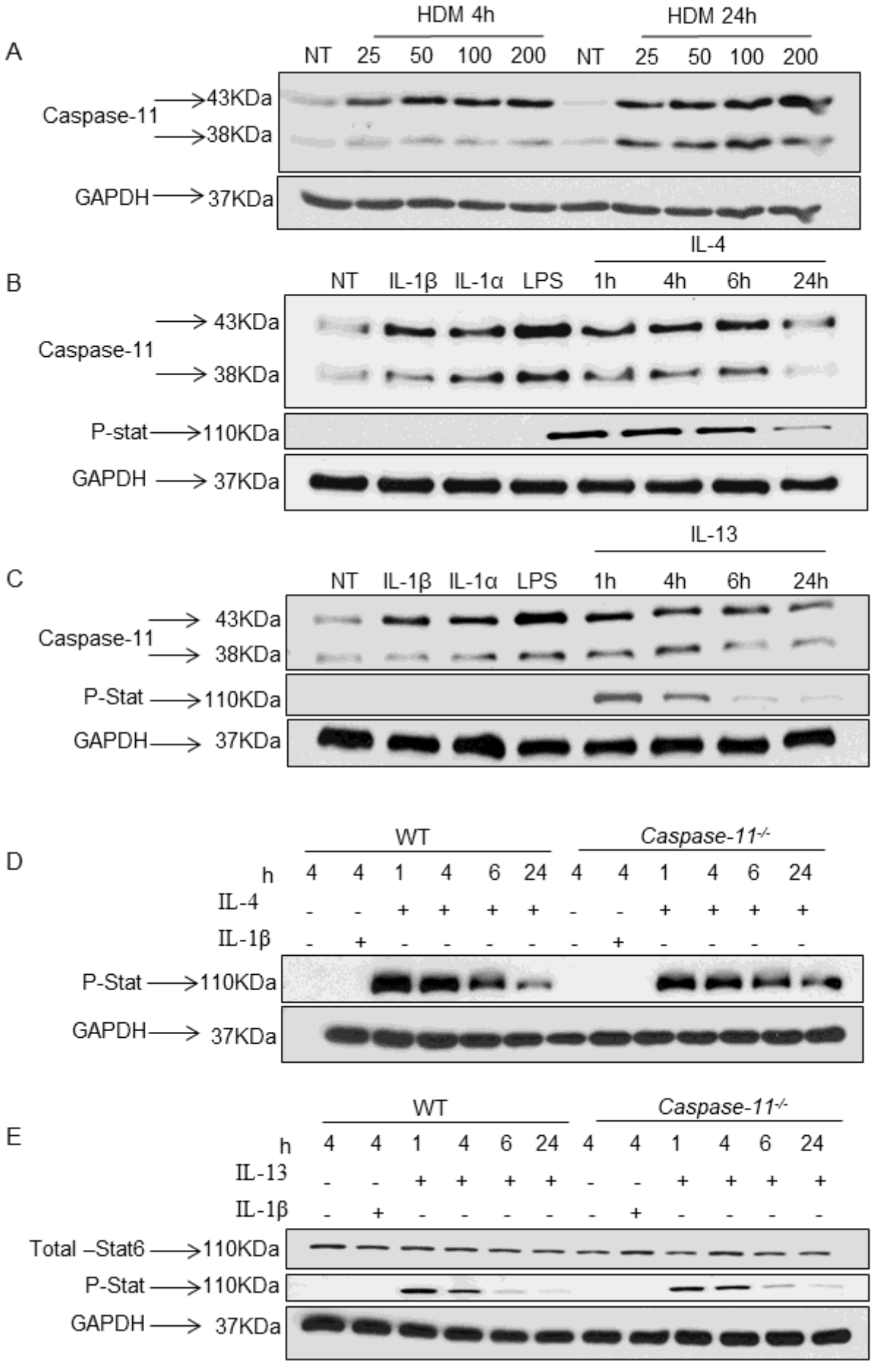

A
HDM 4h
HDM 24h
NT 25 50 100 200 NT 25 50 100 200
Caspase-11
43KDa
38KDa
GAPDH 37KDa
B
IL-4
NT IL-1β IL-1α LPS 1h 4h 6h 24h
Caspase-11
43KDa
38KDa
P-stat 110KDa
GAPDH 37KDa
C
IL-13
NT IL-1β IL-1α LPS 1h 4h 6h 24h
Caspase-11
43KDa
38KDa
P-Stat 110KDa
GAPDH 37KDa
D
WT
Caspase-11-/-
h 4 4 1 4 6 24 4 4 1 4 6 24
IL-4 - - + + + + - - + + + +
IL-1β - + - - - - - + - - - -
P-Stat 110KDa
GAPDH 37KDa
E
WT
Caspase-11-/-
h 4 4 1 4 6 24 4 4 1 4 6 24
IL-13 - - + + + + - - + + + +
IL-1β - + - - - - - + - - - -
Total –Stat6 110KDa
P-Stat 110KDa
GAPDH 37KDa

Figure 1

HDMs, IL-4 and IL-13 cytokines promote caspase-11 expression in macrophages derived from WT mice. WT macrophages were not treated (NT) or treated with different concentrations of HDM (25, 50, 100 and 200µg/ml) for 4 or 24 h. Caspase-11 expression was examined by Western blotting. GAPDH was used as a loading control (A). WT macrophages were treated with several inflammatory stimuli (IL-1β, IL-1α or LPS) or stimulated with different concentrations of IL-4 (5ng/mL) (B) or IL-13 (20ng/mL) (C) for 1, 4, 6 and 24 h and compared to NT samples. Western blot membranes were immunoblotted with capsase-11 or phospho-STAT6 antibodies and GAPDH was used as a loading control. WT or caspase-11-/- macrophages were NT, treated with IL-1β or stimulated with different concentrations of IL-4 (5ng/mL) (D) or IL-13 (20ng/mL) (E) for 1, 4, 6 and 24 h. Membranes were immunoblotted with total Stat6, phospho-STAT6 antibodies, GAPDH was used as a loading control. Two independent experiments were performed and lead to the same results. Representative blots are shown from one of the two experiments (n=2).

Fig.2

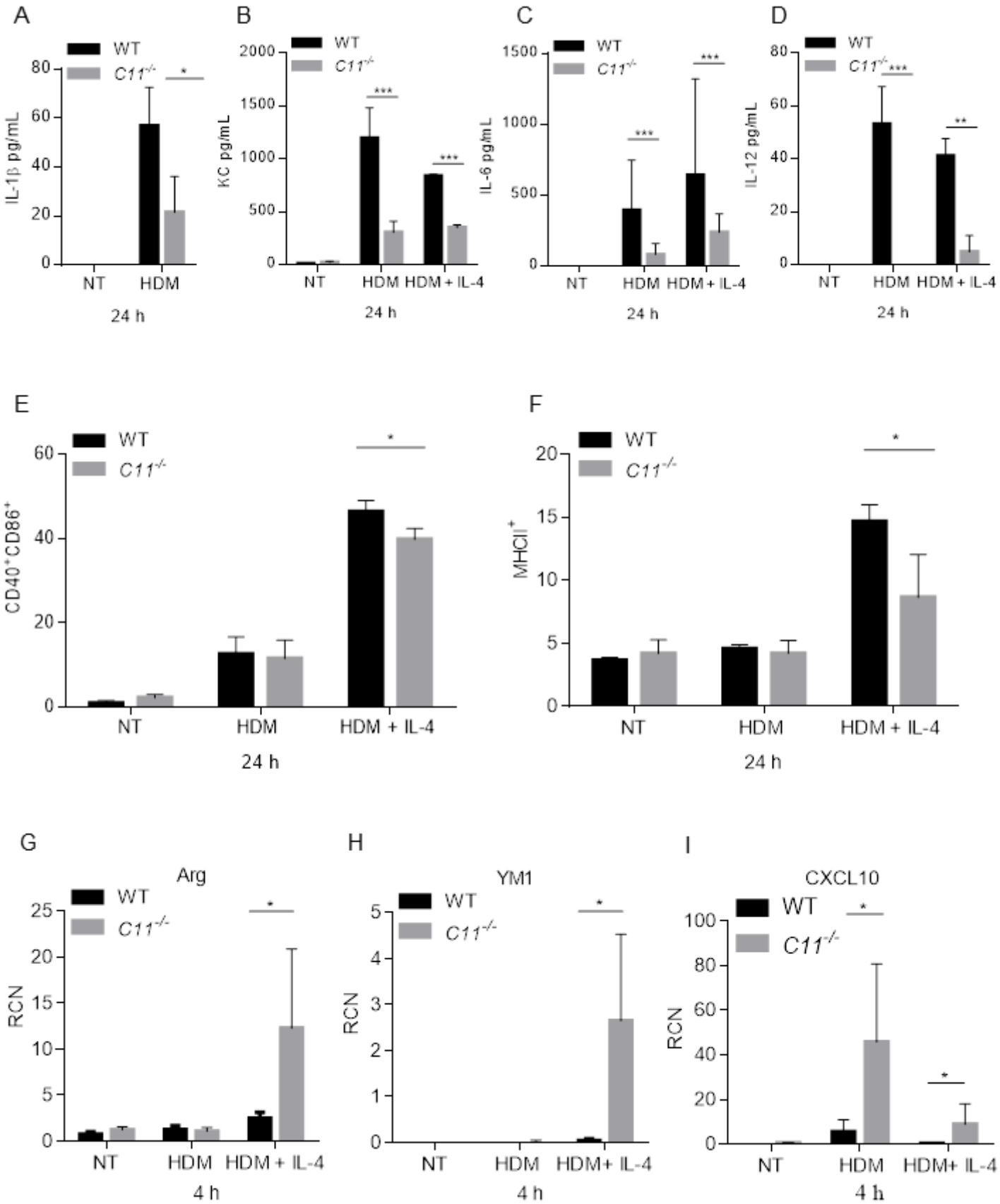
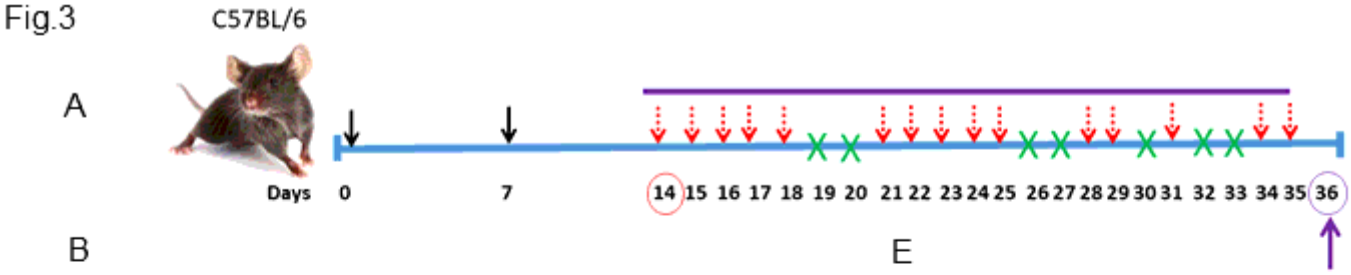
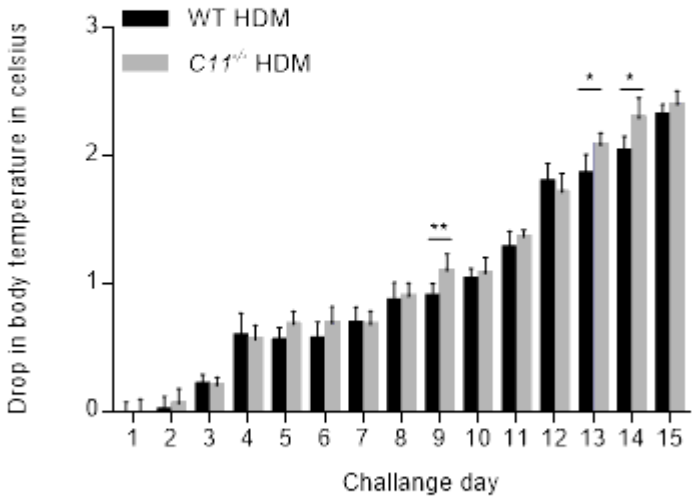

A
B
C
D
WT
C11-/-
IL-1β pg/mL
KC pg/mL
IL-6 pg/mL
IL-12 pg/mL
NT
HDM
HDM + IL-4
24 h
E
F
CD40+CD86+
MHCII+
G
H
I
Arg
YM1
CXCL10
RCN
4 h

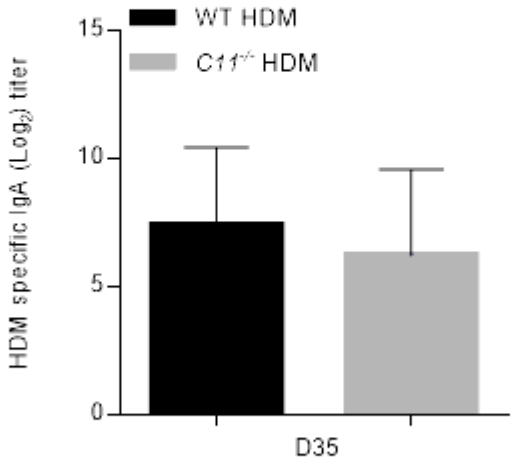
Figure 2

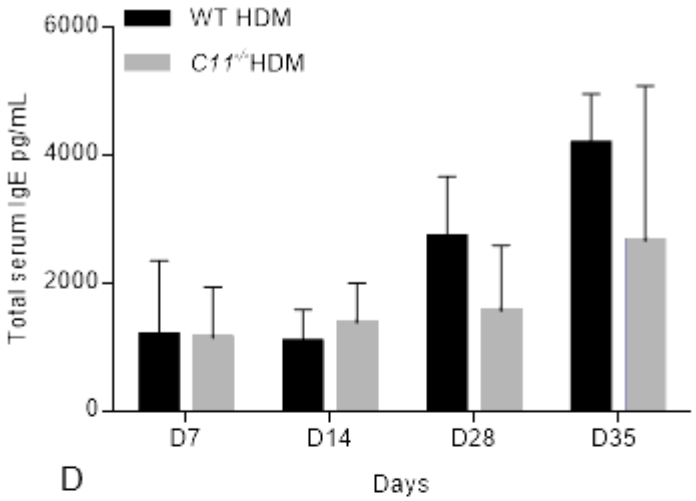
Caspase-11-/- macrophages show reduced release of proinflammatory cytokines and reduced expression of costimulatory molecules post-HDM treatment. Level of IL-1β released in the supernatants of WT or caspase-11-/- macrophages treated with 100μg/mL of HDM 24 h post-treatment (A). Data represent the mean ± SD (n=4) obtained from four independent experiments. Multiple t-tests performed for statistical analysis, * $p < 0.05$. Concentrations of keratinocyte-derived protein chemokine (KC) (B), IL-6 (C), and IL-12 (D) released in supernatants from WT and caspase-11-/- macrophages either un-treated (NT), treated with HDM (100μg/mL), pre-stimulated with IL-4 (5ng/mL) then treated with HDM and IL-4 for 24 h. Data represent the mean ± SD (n=4) obtained from four independent experiments. Multiple t-tests performed for statistical analysis, ** $p < 0.01$, *** $p < 0.001$. Expression of costimulatory molecules CD40 and CD86 (E), and MHCII (F) by flow cytometry 24 h post- HDM treatment. Data represent the mean ± SD (n=3) obtained from three independent experiments. Multiple t-tests performed for statistical analysis, * $p < 0.05$. WT and caspase-11-/- macrophages either NT, treated with HDM (100μg/mL), pre-stimulated with IL-4 (5ng/mL) then treated with HDM and IL-4 for 4 h. Expression of M2 (Arg1, YM1) or M1 (CXCL10) markers by RT-qPCR 4 h post-treatment (G, H & I). RCN on the Y axis represents relative copy number. Data represent the mean ± SD (n=3) obtained from three independent experiments. Multiple t-tests performed for statistical analysis, * $p < 0.05$.

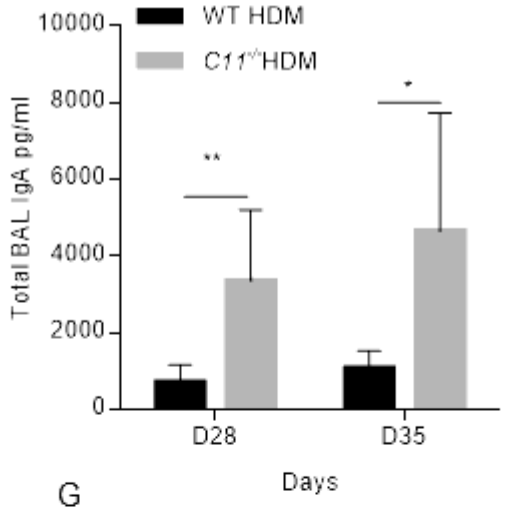
Fig.3

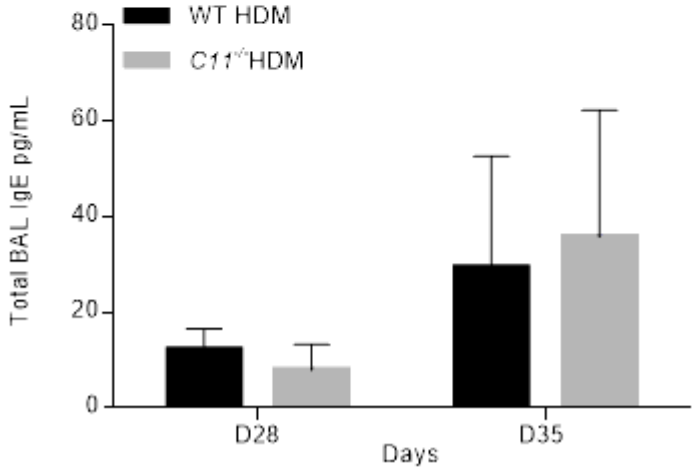

C57BL/6
A
Days
0
7
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36


B
WT HDM
C11-/- HDM
Drop in body temperature in celsius
Challange day
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
0 1 2 3
* * **


E
WT HDM
C11-/- HDM
HDM specific IgA (Log2) titer
D35
0 5 10 15


C
WT HDM
C11-/-HDM
Total serum IgE pg/mL
Days
D7 D14 D28 D35
0 2000 4000 6000


F
WT HDM
C11-/-HDM
Total BAL IgA pg/ml
Days
D28 D35
0 2000 4000 6000 8000 10000
** *


D
WT HDM
C11-/-HDM
Total BAL IgE pg/mL
Days
D28 D35
0 20 40 60 80

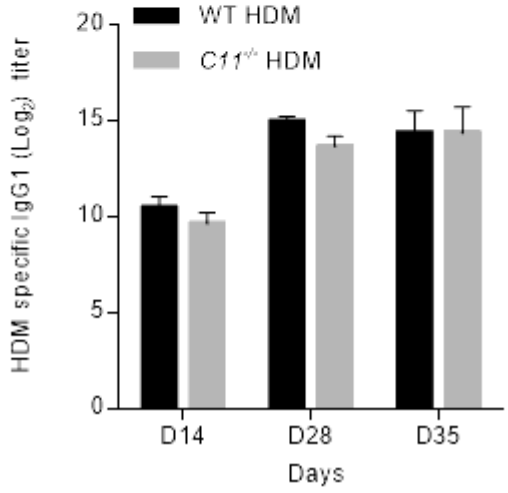

G
WT HDM
C11-/- HDM
HDM specific IgG1 (Log2) titer
Days
D14 D28 D35
0 5 10 15 20

Figure 3

HDM sensitization and challenge promotes differential allergic inflammation and antibody response in live WT and caspase-11-/- mice. Schematic of HDM model (A), five WT and five caspase-11-/- mice were sensitized with alum + 100μg HDM on day 0 and 7 intra-peritoneally (solid arrows). Mice were challenged with 15 doses of HDM intranasally (25μg) as indicated (dashed arrows). Days without a challenge are indicated by (X) (A). The experiment was done twice with seven mice per group in the first in vivo experiment and 5 mice per group in the second in vivo experiment and lead to similar results. Drop in surface body temperature after intra-nasal challenges with HDM was measured before and thirty minutes after each challenge (B). Data indicates the difference in body temperature before and after each challenge. The experiment was done twice with seven mice per group in the first in vivo experiment and 5 mice per group in the second in vivo experiment and lead to similar results. Data represent the mean ± SD (n=5) obtained from the second in vivo experiment. Two-way ANOVA performed for statistical analysis, * $p < 0.05$, ** $p < 0.01$, the experiment was performed twice. Total IgE level was quantified in serum (C) and BAL fluid samples (D) collected before sensitization (day 0), after sensitization with HDM and alum (day 14), day 28, and 24 h after the last challenge (day 35). HDM-specific IgA was quantified on day 28 and 35 (E), and total IgA level was quantified in BAL fluid on day 35 (F). HDM-specific IgG1 was quantified in serum samples collected before sensitization (day 0), after sensitization with HDM and alum (day 14), day 28, and 24 h after the last challenge (day 35) (G). Day 0, 14 and (28, 35) represent naïve, sensitized, and sensitized + challenged mice. The experiment was done twice with seven mice per group in the first in vivo experiment and 5 mice per group in the second in vivo experiment and lead to similar results. Data represent the mean ± SD (n=5) obtained from the second in vivo experiment. The experiment was done twice and lead to similar results. Multiple t-tests performed for statistical analysis, * $p < 0.05$, ** $p < 0.01$, the experiment was performed twice.

Fig.4

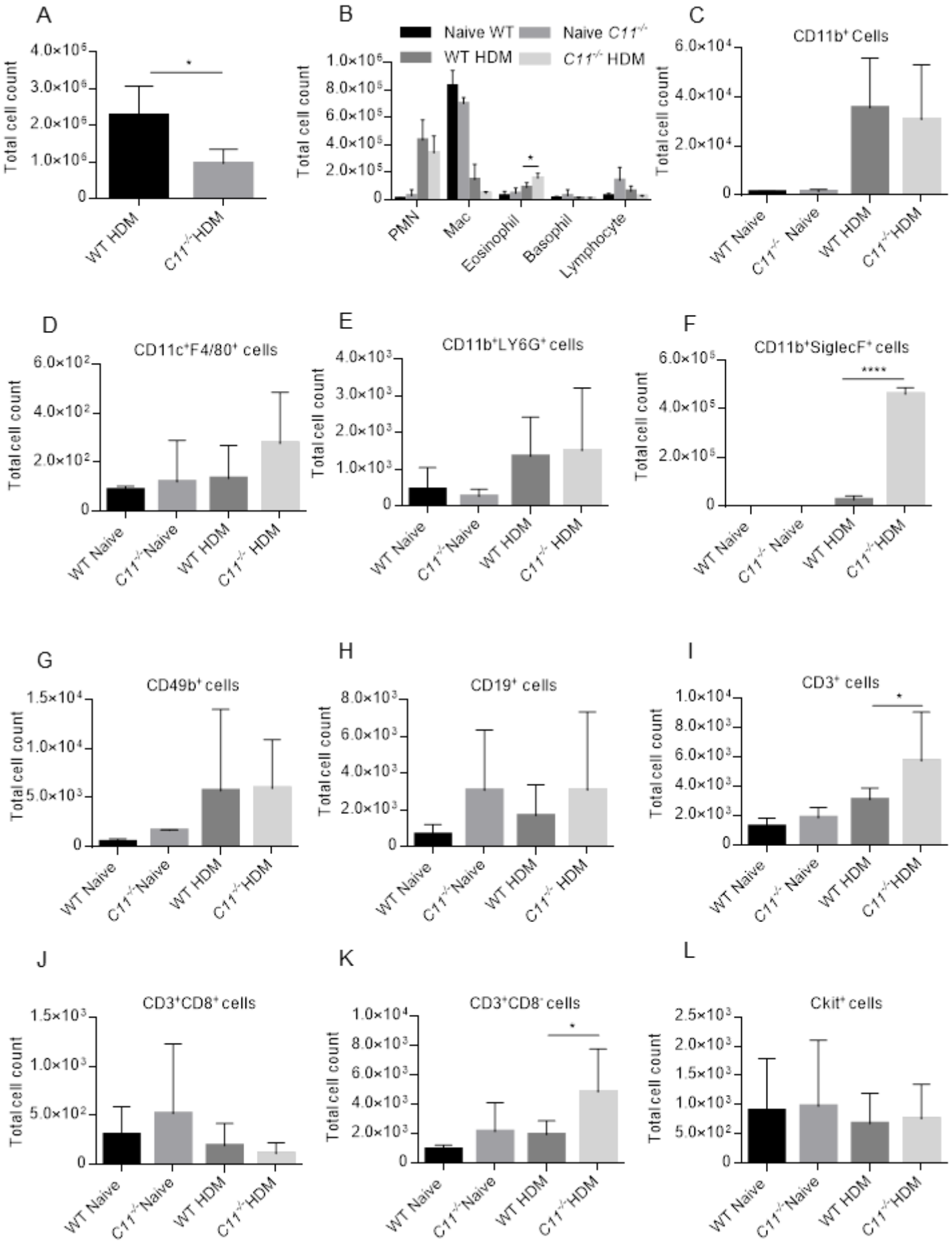

A
Total cell count
4.0×10⁶
3.0×10⁶
2.0×10⁶
1.0×10⁶
0
*
WT HDM
C11⁻/⁻HDM
B
Naive WT
Naive C11⁻/⁻
WT HDM
C11⁻/⁻ HDM
Total cell count
1.0×10⁶
8.0×10⁵
6.0×10⁵
4.0×10⁵
2.0×10⁵
0
*
PMN
Mac
Eosinophil
Basophil
Lymphocyte
C
CD11b⁺ Cells
Total cell count
6.0×10⁴
4.0×10⁴
2.0×10⁴
0
WT Naive
C11⁻/⁻ Naive
WT HDM
C11⁻/⁻HDM
D
CD11c⁺F4/80⁺ cells
Total cell count
6.0×10²
4.0×10²
2.0×10²
0
WT Naive
C11⁻/⁻Naive
WT HDM
C11⁻/⁻ HDM
E
CD11b⁺LY6G⁺ cells
Total cell count
4.0×10³
3.0×10³
2.0×10³
1.0×10³
0
WT Naive
C11⁻/⁻Naive
WT HDM
C11⁻/⁻ HDM
F
CD11b⁺SiglecF⁺ cells
Total cell count
6.0×10⁵
4.0×10⁵
2.0×10⁵
0
****
WT Naive
C11⁻/⁻ Naive
WT HDM
C11⁻/⁻HDM
G
CD49b⁺ cells
Total cell count
1.5×10⁴
1.0×10⁴
5.0×10³
0
WT Naive
C11⁻/⁻Naive
WT HDM
C11⁻/⁻HDM
H
CD19⁺ cells
Total cell count
8.0×10³
6.0×10³
4.0×10³
2.0×10³
0
WT Naive
C11⁻/⁻Naive
WT HDM
C11⁻/⁻ HDM
I
CD3⁺ cells
Total cell count
1.0×10⁴
8.0×10³
6.0×10³
4.0×10³
2.0×10³
0
*
WT Naive
C11⁻/⁻ Naive
WT HDM
C11⁻/⁻HDM
J
CD3⁺CD8⁺ cells
Total cell count
1.5×10³
1.0×10³
5.0×10²
0
WT Naive
C11⁻/⁻Naive
WT HDM
C11⁻/⁻HDM
K
CD3⁺CD8⁻ cells
Total cell count
1.0×10⁴
8.0×10³
6.0×10³
4.0×10³
2.0×10³
0
*
WT Naive
C11⁻/⁻Naive
WT HDM
C11⁻/⁻HDM
L
Ckit⁺ cells
Total cell count
2.5×10³
2.0×10³
1.5×10³
1.0×10³
5.0×10²
0
WT Naive
C11⁻/⁻Naive
WT HDM
C11⁻/⁻HDM

Figure 4

WT and caspase-11-/- mice show differential cellular infiltration in their BAL fluid in response to HDM. Immune cells in bronchial alveolar lavage (BAL) fluids of WT or caspase-11-/- mice sensitized and challenged with HDM. Twenty-four hours following the last HDM challenge, cells were isolated by centrifugation and counted with a hemocytometer (A). Total BAL fluid cells were spotted on the cytospin chamber, stained and counted (B). The experiment was done twice with seven mice per group in the first in vivo experiment and 5 mice per group in the second in vivo experiment and lead to similar results. Data represent the mean ± SD (n=4) per group obtained from the second in vivo experiment. Multiple t-tests performed for statistical analysis, * $p < 0.05$. Specific cell populations in the BAL fluids were determined by staining cells with several markers followed by flow cytometry. Flow markers are (CD11b+), (CD11c+F480+), (CD11b+Ly6G+), (CD11b+SiglecF+), (CD49b+), (CD19+), CD3+, (CD3+CD8+), (CD3+CD8-) and (C-kit+) (C-L). The experiment was done twice with seven mice per group in the first in vivo experiment and 5 mice per group in the second in vivo experiment. Data represent the mean ± SD (n=9) per group pooled from the two experiments. Multiple t-tests performed for statistical analysis, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig.5

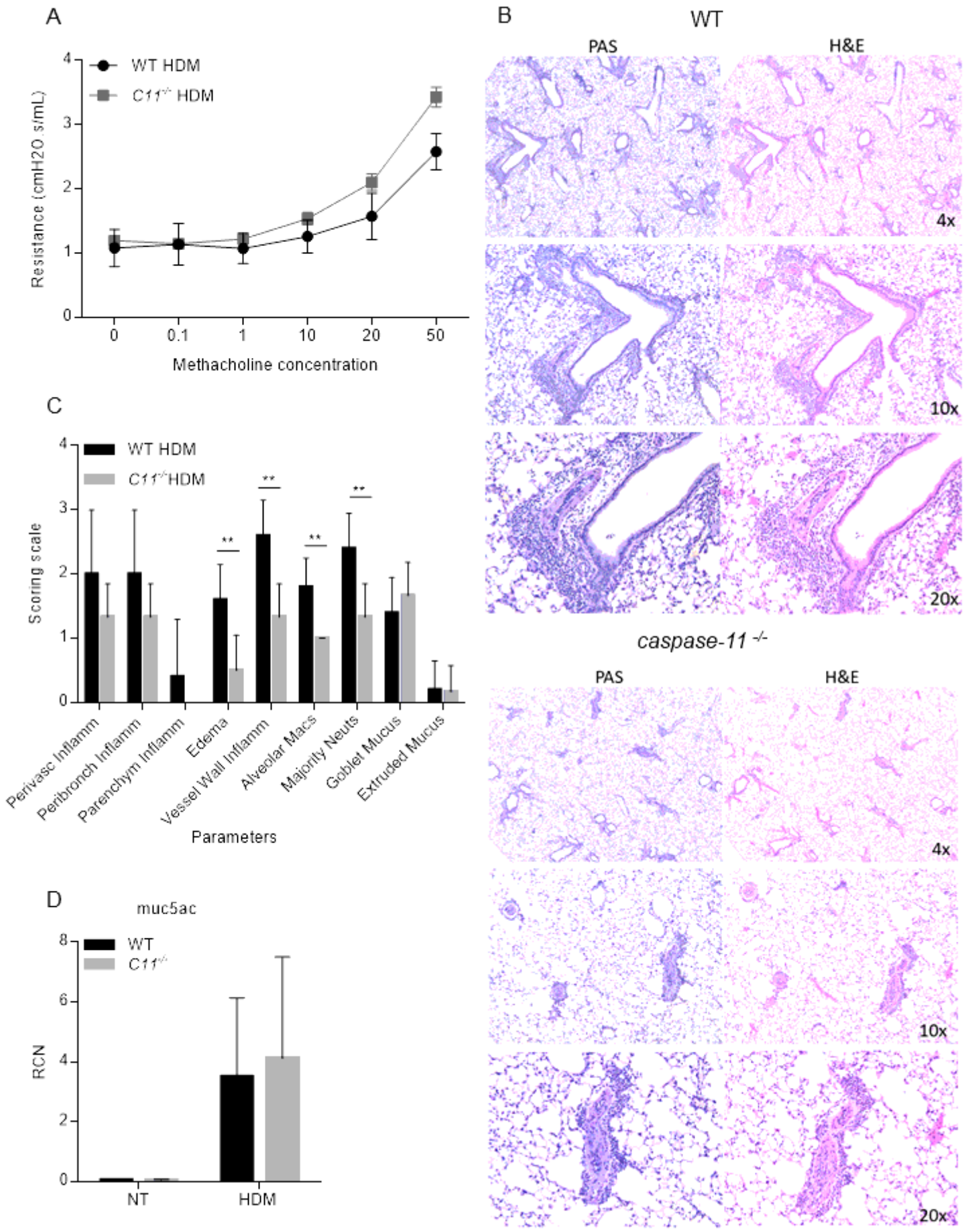

A
WT HDM
C11-/- HDM
Resistance (cmH2O s/mL)
Methacholine concentration
B
WT
PAS
H&E
4x
10x
20x
caspase-11 -/-
C
WT HDM
C11-/-HDM
Scoring scale
Perivasc Inflamm
Peribronch Inflamm
Parenchym Inflamm
Edema
Vessel Wall Inflamm
Alveolar Macs
Majority Neuts
Goblet Mucus
Extruded Mucus
Parameters
D
muc5ac
WT
C11-/-
RCN
NT
HDM

Figure 5

Caspase-11-/- mice show reduced lung inflammation with similar airway resistance and mucus production. Airway hyper-responsiveness to increasing concentrations of methacholine was measured 24 h after the last HDM challenge. Airway hyper-responsiveness was measured by recording changes in lung resistance of WT and caspase-11-/- mice (A). The experiment was performed once with five mice per genotype. Data represent the mean ± SD (n=5) per group obtained from one experiment. Two-way ANOVA performed for statistical analysis. A linear mixed model was used to take account of the correlation among observations from the same mouse within the same cage. No significant difference was seen between caspase-11-/- and WT on the lung contraction averaged across the drug concentrations. Representative sections showing reduced lung inflammation in caspase-11-/- mice exposed to HDM compared to WT (B). The experiment was done twice with seven mice per group in the first in vivo experiment and 5 mice per group in the second in vivo experiment. Representative images are shown from the second in vivo experiment. Lung sections were formalin-fixed, stained with periodic acid Schiff reagent (PAS) or H& E. Magnifications are 4x, 10x and 40x. Quantification of inflammation and cellular infiltration in the lungs scored from images were done in a blinded fashion by a pathologist of the Comparative Pathology and Mouse Phenotyping Shared Resource at the Ohio State University (C). Data represent the mean ± SD (n=5) per group obtained from the second in vivo experiment. Multiple t tests performed for statistical analysis, * $P < 0.05$. Representative (n=2) expression of muc5ac mRNA was measured by quantitative PCR obtained from the second in vivo experiment (D).

Fig.6

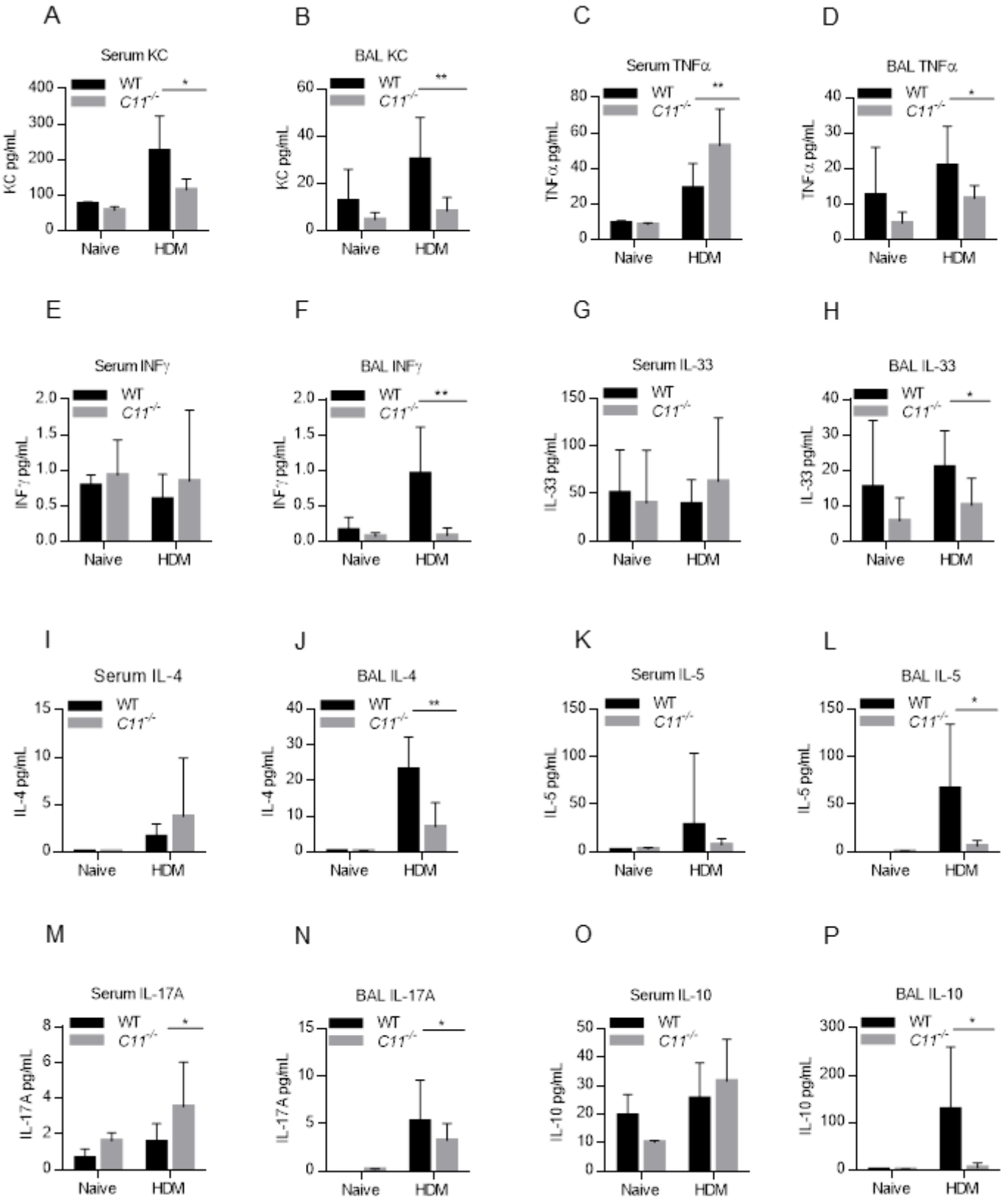

A
Serum KC
KC pg/mL
400
300
200
100
0
WT
C11-/-
*
Naive
HDM
B
BAL KC
KC pg/mL
60
40
20
0
WT
C11-/-
**
Naive
HDM
C
Serum TNFα
TNFα pg/mL
80
60
40
20
0
WT
C11-/-
**
Naive
HDM
D
BAL TNFα
TNFα pg/mL
40
30
20
10
0
WT
C11-/-
*
Naive
HDM
E
Serum INFγ
INFγ pg/mL
2.0
1.5
1.0
0.5
0.0
WT
C11-/-
Naive
HDM
F
BAL INFγ
INFγ pg/mL
2.0
1.5
1.0
0.5
0.0
WT
C11-/-
**
Naive
HDM
G
Serum IL-33
IL-33 pg/mL
150
100
50
0
WT
C11-/-
Naive
HDM
H
BAL IL-33
IL-33 pg/mL
40
30
20
10
0
WT
C11-/-
*
Naive
HDM
I
Serum IL-4
IL-4 pg/mL
15
10
5
0
WT
C11-/-
Naive
HDM
J
BAL IL-4
IL-4 pg/mL
40
30
20
10
0
WT
C11-/-
**
Naive
HDM
K
Serum IL-5
IL-5 pg/mL
150
100
50
0
WT
C11-/-
Naive
HDM
L
BAL IL-5
IL-5 pg/mL
150
100
50
0
WT
C11-/-
*
Naive
HDM
M
Serum IL-17A
IL-17A pg/mL
8
6
4
2
0
WT
C11-/-
*
Naive
HDM
N
BAL IL-17A
IL-17A pg/mL
15
10
5
0
WT
C11-/-
*
Naive
HDM
O
Serum IL-10
IL-10 pg/mL
50
40
30
20
10
0
WT
C11-/-
Naive
HDM
P
BAL IL-10
IL-10 pg/mL
300
200
100
0
WT
C11-/-
*
Naive
HDM

Figure 6

Serum and BAL from caspase-11-/- mice show differential levels of Th1 and Th2 cytokines compared to WT. Sera and BAL fluid samples were obtained from mice on day 35 post-HDM challenge. Level of serum and BAL fluid cytokines were quantified my multiplex MSD electrochemiluminesence (OSU core facility). Day 0 represents serum obtained from naïve animals, while day 35 represents serum obtained from animals exposed to HDM for 35 days. Serum and BAL KC (A & B), TNFα (C & D), INFγ (E & F), IL-33 (G & H), IL-4 level (I & J), IL-5 (K & L), IL-17A (M & N), IL-10 level (O & P). Data represent the mean ± SD (n=4) per group obtained from the second in vivo experiment. Multiple t tests performed for statistical analysis, * $P < 0.05$, ** $p < 0.01$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AbuKhweekscoringrubric.pdf
- Supplementaryfig.1.tif