

General, but not myeloid or type II lung epithelial cell, myeloid differentiation factor 88 deficiency abrogates house dust mite induced allergic lung inflammation

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Summary

Asthma is a highly prevalent chronic allergic inflammatory disease of the airways affecting people worldwide. House dust mite (HDM) is the most common allergen implicated in human allergic asthma. HDM-induced allergic responses are thought to depend upon activation of pathways involving Toll-like receptors and their adaptor protein myeloid differentiation factor 88 (MyD88). We sought here to determine the role of MyD88 in myeloid and type II lung epithelial cells in the development of asthma-like allergic disease using a mouse model. Repeated exposure to HDM caused allergic responses in control mice characterized by influx of eosinophils into the bronchoalveolar space and lung tissue, lung pathology and mucus production and protein leak into bronchoalveolar lavage fluid. All these responses were abrogated in mice with a general deficiency of MyD88 but unaltered in mice with MyD88 deficiency, specifically in myeloid or type II lung epithelial cells. We conclude that cells other than myeloid or type II lung epithelial cells are responsible for MyD88-dependent HDM-induced allergic airway inflammation.

Keywords: asthma, epithelial, HDM, MyD88

Introduction

Asthma is a chronic pulmonary disorder that affects approximately 300 million people globally, and asthma-related symptoms have been shown to be present in 5–16% of the world's population [1,2]. Asthma is characterized by bronchial hyperresponsiveness leading to obstruction and inflammation of the airways, giving rise to symptoms such as wheezing, coughing and chest tightness. This hyperactivity of the bronchi can be triggered by exposure to various stimuli including the most common aeroallergen house dust mite (HDM). Up to 85% of asthmatic patients are allergic to HDM [3].

Allergens such as HDM can be sampled in the airway lumen by dendritic cells (DCs) that have close interactions with airway epithelial cells (AECs). AECs can produce cytokines that activate and cause maturation of DCs that, in turn, trigger T helper type 2 (Th2) cell responses [4]. Although the Th2 cell response is undeniably a crucial hallmark feature of asthma, its activation depends upon innate immune functions of AECs and DCs. Many components of

HDM can contribute to its allergenicity and HDM extracts frequently contain pathogen-associated molecular patterns (PAMPs) that can be detected by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) [3].

While, traditionally, the expression and function of TLRs have been investigated in specialized immune cells, AECs are among the first cells that come into contact with PAMPs entering the airways. Lung epithelial cells express various TLRs and are thereby able to detect and respond to aeroallergens [5,6]. Experiments using mice lacking TLR-4 on either haematopoietic or radioresistant structural cells (including AECs) showed that activation and migration of DCs is regulated by expression of TLR-4 on structural cells, suggesting a primary role for airway epithelium in the initiation of allergic responses [7]. Several types of airway epithelial cells exist that can come into contact with HDM, such as bronchiolar cells and the epithelial cells that form the alveolus, type I and type II alveolar epithelial cells. Of these, type II alveolar type II (which are known for their secretion of surfactant proteins) are of particular interest

because of their role during innate immunity against bacteria [8,9].

Myeloid differentiation factor 88 (MyD88) is an important adaptor protein for all TLRs except TLR-3 [10]. MyD88 mediates TLR-driven signalling pathways, leading eventually to the production of inflammatory cytokines and chemokines. Using an animal model in which mice were exposed repeatedly to HDM to induce an asthma-like allergic disease, cardinal features of allergic asthma such as eosinophilia and Th2 responses were found to be dependent upon MyD88 [11]. To our knowledge, we are the first to investigate the cell-specific role of MyD88 during asthma. For this we made use of the Cre-Lox system to generate myeloid- and type II lung epithelial-specific MyD88 knock-out mice which were then subjected to repeated HDM exposure to mimic allergic asthma.

Material and methods

Animals

MyD88 gene-deficient [knock-out (KO)] mice were provided by Dr S. Akira (Research Institute for Microbial Diseases, Osaka, Japan) and back-crossed at least six times to a C57Bl/6 genetic background. Age- and sex-matched C57Bl/6 control mice were obtained from Harlan Nederland (Horst, the Netherlands). Homozygous *MyD88^{fl/fl}* [12] mice were crossed with lysozyme M (*LysM*) cre mice [13] (obtained from the Jackson Laboratory, Bar Harbor, ME, USA), or surfactant protein C (*Sftpc*) cre mice [14] (kindly provided by B. L. Hogan (Duke University School of Medicine, Durham, NC, USA), to generate myeloid (*LysMcre-MyD88-lox*) and lung epithelial cell (*Sftpccre-MyD88-lox*)-specific MyD88-deficient mice. *MyD88^{fl/fl}* Cre-negative littermates were used as controls. All mice were back-crossed at least six times to a C57Bl/6 background and age- and sex-matched when used in experiments. Animals were housed in standardized specific pathogen-free conditions and all experiments were approved by the Animal Care and Use Committee of the University of Amsterdam.

Harvest of primary cells for genetic characterization of *Sftpccre-MyD88-lox* mice

Macrophages and splenocytes were harvested exactly as described previously [15]. In short, peritoneal lavage was performed with 5 ml sterile phosphate-buffered saline (PBS) under isoflurane anaesthesia and lavage fluid was collected in PBS containing a final concentration of 10% fetal bovine serum (FBS), 1% antibiotics (penicillin, streptomycin and amphotericin B; GIBCO, Paisley, UK); macrophages were seeded in flat-bottomed 96-well cell culture plates (Greiner Bio-one, Alphen a/d Rijn, the Netherlands) at a density of approximately 30 000 per well in RPMI

complete (containing 10% FBS, 1% antibiotics, 10 mM L-glutamine; GIBCO) and left to adhere overnight before harvesting the cells for genetic analysis. To obtain splenocytes, spleens were harvested and first filtered through a 40- μ M filter (BD Falcon, Franklin Lakes, NJ, USA). Erythrocytes in the splenic cell suspension were lysed using ammonium chloride containing lysis buffer, and then in RPMI complete at a density of 500 000 per well in 96-well U-bottomed culture plates (Greiner Bio-one).

Lung epithelial cells were isolated from the mouse lung according to a protocol adapted from Raoust *et al.* [16]. Briefly, mice were euthanized and the trachea was cannulated. Lungs were washed three times with PBS to remove alveolar macrophages. Lungs were then perfused with 10–20 mL PBS supplemented with 1% antibiotics and 1% heparin (5000 IU/mL; LEO Pharma BV, Amsterdam, the Netherlands) injected into the right heart ventricle until lungs were cleared of blood. Two ml of dispase (BD Biosciences, Bedford, MA, USA) were then instilled into the lungs through the cannulated trachea. Lungs were removed and placed into a sterile tube prefilled with 1 ml of dispase and the suspension was incubated for 45 min in a 37°C incubator to speed up enzymatic digestion. After removing visible large bronchi from the lungs suspended in 4 ml of F12K nutrient mixture (Kaighn's modification; Invitrogen, Carlsbad, CA, USA) with 0.01% DNase I (Sigma, St Louis, MO, USA) and 1% antibiotics solution, the cell isolate was filtered through nylon mesh filters (sizes 100, 70 and 40 μ M; BD Falcon). The obtained cell suspension was then centrifuged at 400 *g* for 10 min at 4°C to collect the cell pellet, which was resuspended in F12K medium supplemented with 2% FCS, 1% antibiotics solution and 2 mM L-glutamine. To purify lung epithelial cells further, cells were sorted on a fluorescence activated cell sorter (FACS) Aria II cell sorting machine (BD Biosciences), and the CD45⁺Epcam⁺ cells were identified as lung epithelial cells. Staining antibodies were purchased from eBioscience (San Diego, CA, USA).

Quantitative polymerase chain reaction (PCR)

The deletion efficiency of MyD88 was determined after extracting DNA from purified macrophages, splenocytes and lung epithelial cells using the Nucleospin Blood Kit (Machery Nagel, Düren, Germany). The residual amount of the 'floxed' region of MyD88 in primary cells of *Sftpccre-MyD88-lox* and littermate control mice was quantified using the primer sequences 5'-ACGCCGGAAC TTTTCGAT-3' (forward); 5'-TTTCTCAATTAGCTCGC TGG-3' (reverse) normalized to unaffected *Socs-3* gene with the primer sequences 5'-ACCTTTCTTATCCGCGAC AG-3' (forward) and 5'-TGCACCAGCTTGAGTACAC AG-3' (reverse) in a SybrGreen reaction on a LightCycler System (LC480; Roche Applied Science, Mannheim, Germany). The amount of remaining 'floxed' MyD88

region was calculated using the 2-delta Ct ($\Delta\Delta C_t$) method, using the amount of genomic DNA from littermate mice for the no-deletion control. The deletion efficiency was calculated as $(1 - \text{residual } MyD88^{\Delta I/I}) \times 100$.

HDM-induced mouse asthma model

HDM allergen whole body extract (Greer Laboratories, Lenoir, NC, USA), derived from the common European HDM species *Dermatophagoides pteronyssinus* (Der p), was used to induce allergic lung inflammation as described previously [17]. Briefly, mice were inoculated intranasally on days 0, 1 and 2 with 25 µg HDM (sensitization phase) and on days 14, 15, 18 and 19 with 6.25 µg HDM (challenge phase). Controls received isotonic sterile saline intranasally on each occasion. Inoculum volume was 20 µl for every HDM and saline exposure and inoculation procedures were performed during isoflurane inhalation anaesthesia. The experiment was ended at day 21 by euthanizing the mice and the subsequent collection and processing of samples: in one experiment bronchoalveolar lavage fluid (BALF) and heparinized blood was collected, in a separate experiment one lung was obtained for pathology and one lung for homogenization, using procedures as described in Daan de Boer *et al.* [17] and below.

Bronchoalveolar lavage and tissue handling

BALF was harvested after exposing the trachea through a midline incision and instilling and retrieving 1 ml of sterile saline 0.9% (in 500-µl aliquots). Cell counts were determined for each BALF sample in a haemocytometer (Beckman Coulter, Fullerton, CA, USA) and differential cell counts by cytospin preparations stained with Giemsa stain (Diff-Quick; Dade Behring AG, Düringen, Switzerland).

Pathology

Lungs were embedded in paraffin after fixation in 10% formalin; 4-µm-thick sections of the area that contained the full-sized lung were stained with haematoxylin and eosin. Pathology and periodic acid Schiff (PAS) was scored (in a double-blinded manner) by an experienced pathologist unaware of the treatment or genotype of the mice. Parameters of allergic lung inflammation were: interstitial inflammation, peribronchial inflammation, oedema and endothelialitis, each graded on a scale of 0–4 (0: absent, 1: mild, 2: moderate, 3: severe, 4: very severe). The total pathology score was expressed as the sum of the score for all parameters [17]. PAS-D staining for carbohydrates in mucus was performed to quantify the amount of mucus. The amount of mucus per lung section was scored in a semiquantitative fashion on a scale from 0 to 8 (0–4 for plug formation, 0–4 for mucus extent) [17]. Eosinophil staining was performed using a monoclonal antibody against major basic protein (MPB; kindly provided by Dr Nancy Lee and Professor James Lee, Mayo Clinic,

Arizona, Scottsdale, AZ, USA). Entire sections were digitized with a slide scanner using the $\times 10$ objective (Olympus dotSlide, Tokyo, Japan). Images were exported in the Tagged Image File Format (TIFF) for quantification. Influx of eosinophils was determined by measuring the MBP immunopositive area by digital image analysis (ImageJ 1.46; National Institute of Health, Bethesda, MD, USA), and expressed subsequently as a percentage of the total lung area. The average of 10 pictures was used for analysis of eosinophilic pulmonary influx [17].

Immunoglobulin (Ig)M assay

Total IgM in BALF was measured using rat anti-mouse IgM as a capture antibody, biotinylated goat anti-mouse IgM as a detection antibody and purified mouse IgM as standard (all from SouthernBiotech, Birmingham, AL, USA).

Statistical analysis

Values are expressed as mean $\pm$ standard deviation (s.e.). Differences between groups were tested by Mann–Whitney *U*-test. GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA) was used for all analyses. Values of $P < 0.05$ were considered statistically significant.

Results

General, but not myeloid or lung epithelial, MyD88 deficiency prevents HDM-induced eosinophil influx

To confirm that MyD88 is indispensable for HDM induced allergic airway inflammation [11], we exposed MyD88KO and control mice to repeated intranasal inoculation of HDM and determined cell influx in the lungs. As shown in Fig. 1a, exposure to HDM but not to saline led to an increased cell influx in control mice, whereas MyD88KO mice did not respond to HDM ($P < 0.001$) and showed cell numbers comparable to those found in saline controls. The predominant cell types found in BALF of control mice were eosinophils, which could not be detected in BALF of MyD88KO mice (Fig. 1b), corroborating previous results reported by others [11]. Moreover, because of the absent migration of granulocytes into the alveolar space, most cells that could be found in BALF of MyD88KO mice were identified as (most probably resident) macrophages (Fig. 1b). To determine a more cell-specific role for MyD88, we made use of the Cre LoX system to generate mice deficient for MyD88 in myeloid cells (LysMcre-MyD88-lox mice) or in alveolar epithelial cells (SftpcCre-MyD88-flox mice). Previously, we have demonstrated that the *MyD88* ^{$\Delta I/I$} allele was deleted in 83.5% of macrophages and in only 9.6% and 19.1% of splenocytes and lung epithelial cells, respectively, isolated from LysMcre-MyD88-lox mice [15]. As expected, the efficiency of cre-induced MyD88 deletion in SftpcCre-

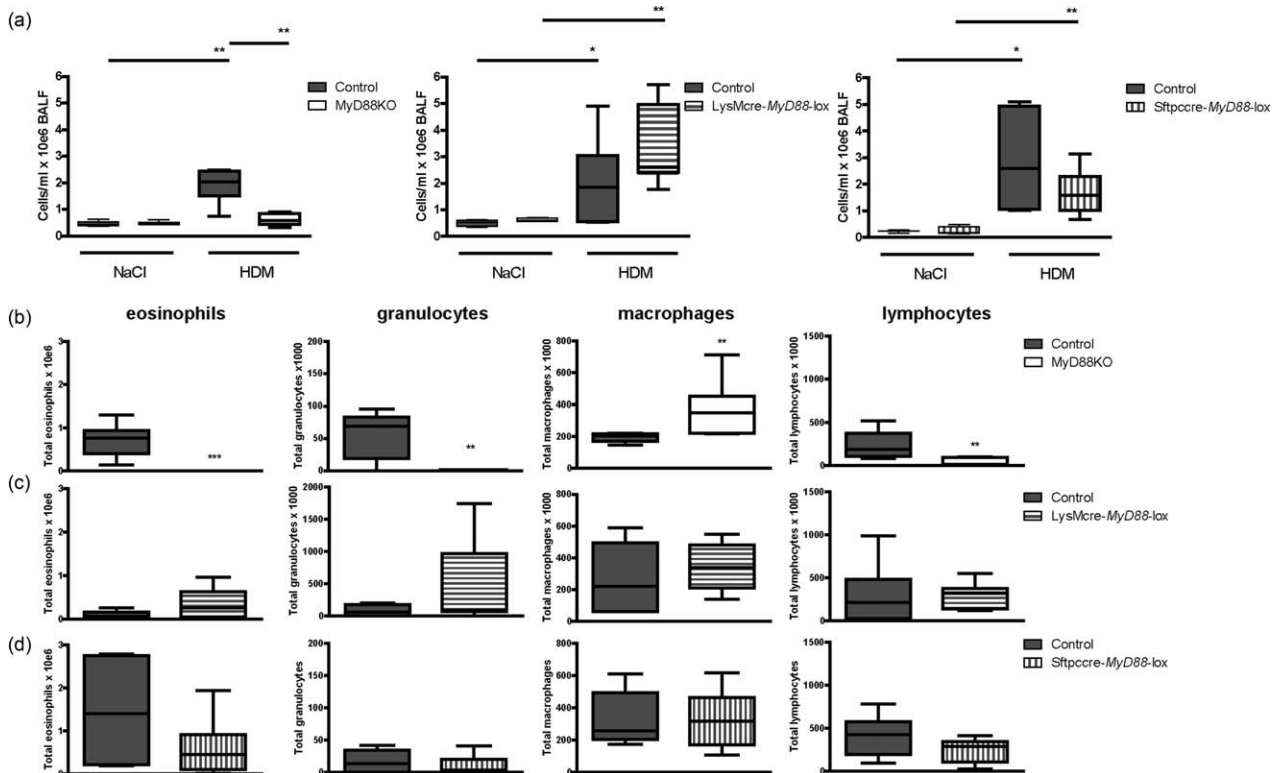


Fig. 1. General, but not myeloid or type II lung epithelial cell, myeloid differentiation factor 88 (MyD88) deficiency abrogates house dust mite (HDM)-induced eosinophil influx into the bronchoalveolar space. MyD88 knock-out (KO) (clear bars), LysMcre-MyD88-lox (horizontally striped bars) and Sftpcrcr-MyD88-lox (vertically striped bars) mice were exposed to either saline ($n = 4$ per group) or HDM ($n = 7-8$ per group) and compared to their matching controls (grey bars). (a) Total cell influx in bronchoalveolar lavage fluid (BALF) in saline (NaCl) or HDM-exposed mice. (b-d) Total number of eosinophils, granulocytes, macrophages and lymphocytes in BALF of mice exposed to HDM. Data are expressed as box-and-whisker diagrams depicting the smallest observation, lower quartile, median, upper quartile and largest observation. Total cells of mutant mice were compared to their matching control mice using the Mann-Whitney U -test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Data are representative of at least two independent experiments.

MyD88-flox mice was very high in isolated lung epithelial cells (82.7%), whereas only 11.7% deletion was observed in splenocytes and no deletion in macrophages (Supporting information, Table S1). When treated with HDM, absence of MyD88 in either myeloid or epithelial cells could not reproduce the results obtained with MyD88KO mice, i.e. total cell and eosinophil influx of both LysMcre-MyD88-lox and Sftpcrcr-MyD88-lox mice was similar to that found in their respective controls (Fig. 1a-c). We also determined eosinophil influx into lung tissue by staining for MBP. As illustrated in Fig. 2a, the percentage of MBP-positive lung surface was significantly lower in MyD88KO mice compared to control mice ($P < 0.05$). However, HDM exposure of lungs of LysMcre-MyD88-lox mice resulted in even slightly more accumulation of tissue eosinophils when compared to littermate controls, while the percentage of MBP staining did not differ between Sftpcrcr-MyD88-lox mice and their controls (Fig. 2b,c). Thus, although these results clearly confirm an essential role for MyD88 in the migration of eosinophils into the alveolar space and lung tissue as a reaction to HDM exposure, neither myeloid nor

epithelial expression of this adaptor protein is responsible for MyD88-dependent eosinophil influx.

General, but not myeloid or lung epithelial, MyD88 deficiency reduces HDM-induced lung pathology

The HDM model used here is characterized by allergic inflammation eventually leading to pulmonary pathological features such as perivascular inflammation, interstitial inflammation, peribronchitis and oedema [17-19]. Using a semiquantitative scoring system to quantify lung pathology as a result of HDM exposure, we found that MyD88KO mice developed significantly less lung inflammation compared to control mice (Fig. 3a, $P < 0.01$). However, MyD88 expression in myeloid or lung epithelial cells did not influence HDM-induced lung pathology (Fig. 3b,c). In accordance with these histological data, protein leak into the alveolar compartment, as measured by BALF IgM concentrations, was attenuated strongly in MyD88KO, but not in LysMcre-MyD88-lox or Sftpcrcr-MyD88-lox mice (Fig. 3d).

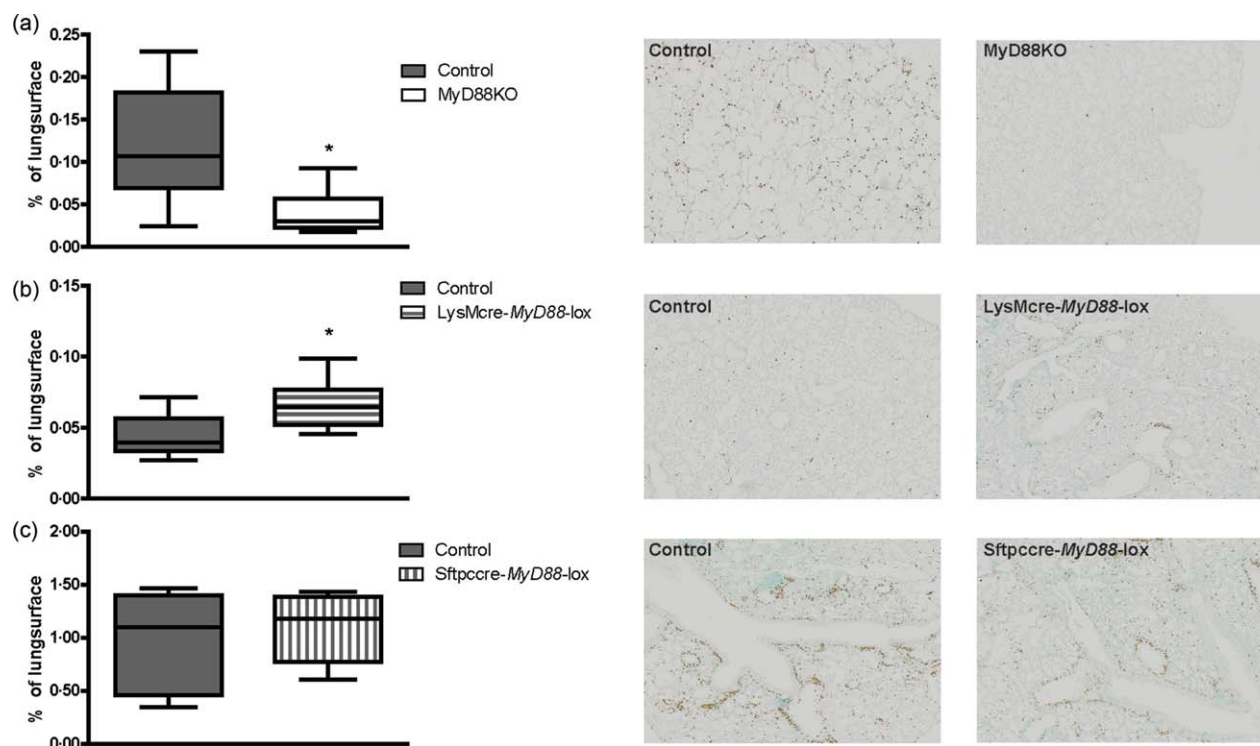


Fig. 2. General, but not myeloid or type II lung epithelial cell, myeloid differentiation factor 88 (MyD88) deficiency abrogates house dust mite (HDM)-induced eosinophil influx into lung tissue. Lung tissue of MyD88 knock-out (KO) (clear bars, a), LysMcre-MyD88-lox (horizontally striped bars, b) and Sftpcrcr-MyD88-lox (vertically striped bars, c) mice ($n = 7-8$ per group) exposed to HDM was stained for major basic protein (MBP) to visualize tissue eosinophils. Representative slides are shown of matching control and transgenic mice on the right of each graph. Graphs show the percentage of lung surface stained positive for eosinophil MBP of mutant mice compared to their matching controls (grey bars) using the Mann-Whitney *U*-test. Data are expressed as box-and-whisker diagrams depicting the smallest observation, lower quartile, median, upper quartile and largest observation. * $P < 0.05$. [Colour figure can be viewed at wileyonlinelibrary.com]

General, but not myeloid or lung epithelial, MyD88 deficiency attenuates airway mucus formation caused by HDM exposure

Lung tissue slides were stained for PAS-D and scored for mucus production (Fig. 4). Whereas HDM induced significant mucus production in control mice, this was reduced strongly in MyD88KO mice (Fig. 4a, $P < 0.05$). Mucus production was not different between LysMcre-MyD88-lox, Sftpcrcr-MyD88-lox and their respective control mice (Fig. 4b,c), indicating further that MyD88 expression in cells other than myeloid or epithelial is crucial for development of HDM-induced airway inflammation.

Discussion

Asthma is a widespread chronic disease that causes significant morbidity and mortality worldwide [1,2]. Traditionally, asthma is considered to be a T lymphocyte-mediated disease. Recently, however, the innate immune host response has been implicated in the pathophysiology of asthma. TLR-4 and MyD88 have been reported to be crucial for the development of asthma, and several studies

have suggested that activation of lung epithelial cells via TLR-mediated detection of inhaled allergens triggers responses that lead eventually to activation of DCs and T cell immunity [7,11,20]. We are the first to use cell-specific conditional KO mice to study the role of MyD88 in a murine asthma model. Although HDM-challenged MyD88KO mice showed no signs of allergic inflammation, mice with specific deletion of MyD88 in myeloid or lung epithelial cells developed allergic lung inflammation in the same manner as their littermate controls.

Our results confirm an earlier study reporting that MyD88 is indispensable for the development of experimentally induced asthma [11]. Indeed, in our model, general expression of MyD88 was crucial for HDM-induced eosinophil influx, lung pathology and mucus formation, all considered hallmarks of allergic asthma. Many cells express MyD88, of which myeloid cells such as macrophages are prominent [21,22]. Pulmonary macrophages have been implicated in allergic airway diseases and have been suggested to be responsible at least in part for preserving inflammation and inflammation associated lung damage as seen during asthma [23]. None the less, LysMcre-MyD88-lox mice demonstrated an unaltered response to HDM

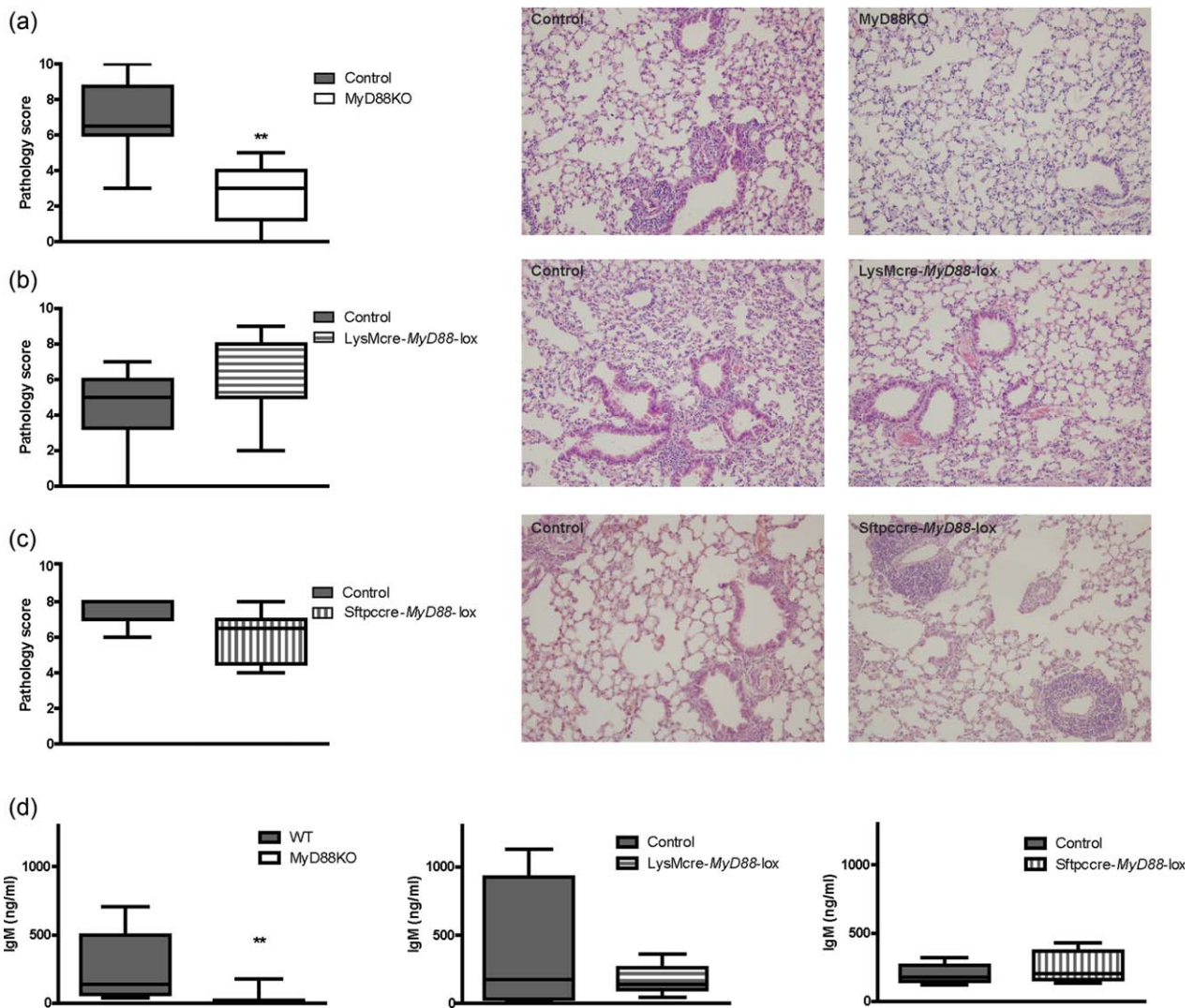


Fig. 3. General, but not myeloid or type II lung epithelial cell, myeloid differentiation factor 88 (MyD88) deficiency attenuates house dust mite (HDM)-induced lung pathology and protein leak. Lung tissue of MyD88 knock-out (KO) (clear bar, a), LysMcre-MyD88-lox (horizontally striped bar, b) and Sftpcrre-MyD88-lox (vertically striped bar, c) mice ($n = 7-8$ per group) exposed to HDM was graded for interstitial and peribronchial inflammation, oedema, endothelialitis and pleuritis. The total pathology score was expressed as the sum of the score of all in panels. (a-c) The scores depicted in diagrams (left) and representative lung histology slides (right). (d) Immunoglobulin (Ig)M concentrations in bronchoalveolar lavage fluid (BALF) of HDM-treated mice to assess protein leak into the alveolar compartment. Data are expressed as box-and-whisker diagrams depicting the smallest observation, lower quartile, median, upper quartile and largest observation. ** $P < 0.01$. [Colour figure can be viewed at wileyonlinelibrary.com]

when compared with their littermate controls, suggesting a non-important role of macrophage MyD88 during asthma. Indeed, cre-mediated deletion of floxed *MyD88* driven by the LysM promoter has been shown to result in high deletion efficiency in macrophages, whereas deletion of MyD88 is only partial in CD11C⁺ DCs [13]. This implies that the role of airway macrophages in regulating inflammatory responses during asthma is not mediated via MyD88-dependent signalling [23].

Airway epithelium lines the airways and provides a natural physical barrier to inhaled substances and helps to remove potentially hazardous matter by means of ciliary

clearance. However, it has become more evident that epithelial cells possess far more features and are capable of detecting and mounting immune responses on their own [5,24-26]. As lung epithelial cells are the first cells that come into contact with allergens such as HDM, it has been proposed that these cells are important regulators of the inflammatory processes responsible for asthmatic disease [20,27]. Evidence for involvement of airway epithelial cells in the pathogenesis of asthma is provided by a study in which bone marrow chimeras that lack expression of TLR-4 on radioresistant structural cells were unable to mount HDM-induced inflammatory responses [7]. In the present

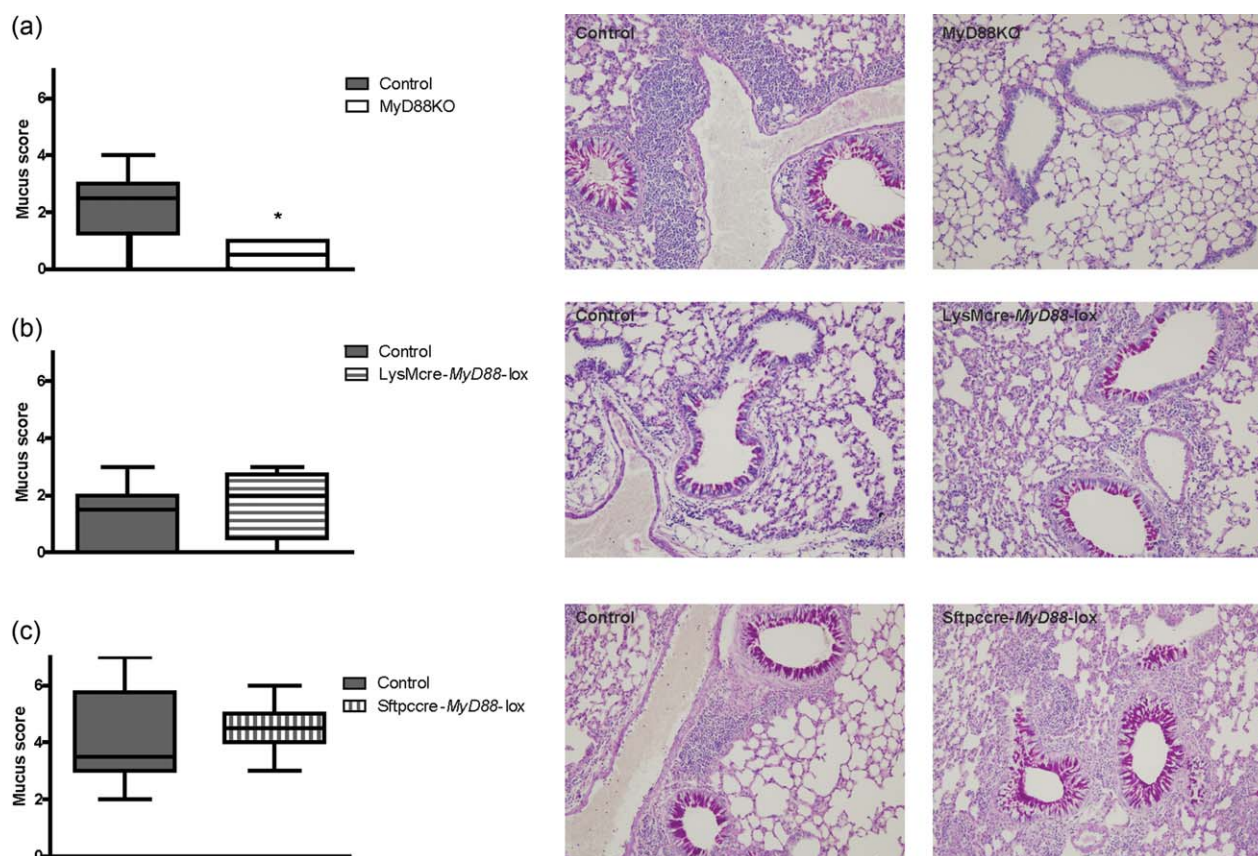


Fig. 4. General, but not myeloid or type II lung epithelial cell, myeloid differentiation factor 88 (MyD88) deficiency attenuates house dust mite (HDM)-induced airway mucus formation. Lung tissue slides stained for periodic acid Schiff (PAS)-D and scored for mucus production of MyD88 knock-out (KO) (clear bar, a), LysMcre-MyD88-lox (horizontally striped bars, b) and Sftpcrcr-MyD88-lox (vertically striped bar, c) mice ($n = 7-8$ per group), after repeated exposure to HDM. Results are compared to matching controls (grey bars). Data are expressed as box-and-whisker diagrams depicting the smallest observation, lower quartile, median, upper quartile and largest observation. Representative slides are shown of matching control and transgenic mice on the right of each graph. * $P < 0.05$. [Colour figure can be viewed at wileyonlinelibrary.com]

study we used the Cre-lox system to generate mice deficient for MyD88 in surfactant protein C producing (type II) epithelial cells [28] to show that these mice were able to respond to repeated HDM exposure. Lack of MyD88 in these lung epithelial cells did not prevent these mice from developing eosinophil influx and lung pathology in response to HDM. These results do not exclude a role of MyD88 in respiratory epithelial cells in general. For example, bronchial epithelial cells, which do not express surfactant and therefore are not deficient for MyD88 in Sftpcrcr-MyD88-lox mice [29], may mediate the MyD88-dependent effect in HDM-induced airway inflammation. This might explain previous results supporting a role for lung epithelial cell TLR-4-mediated responses to HDM [7].

Across experiments, littermate control mice showed variation in some of their responses to HDM. This can be explained, at least in part, by the fact that specific control mice were used for each transgenic mouse type to resemble the genetic profile as much as possible. Moreover, genetically modified mice and corresponding (control) littermates

were always compared in parallel in the same experiment, thereby excluding potential bias introduced by variations in HDM responses between separate experiments.

Our laboratory studied LysMcre-MyD88-lox and Sftpcrcr-MyD88-lox previously in models of bacterial pneumonia, using pathogens that, at least in part, trigger innate immune responses by TLR-MyD88 signalling [15,30]. These investigations showed that the immune response to *Klebsiella pneumoniae* (which signals mainly via TLR-4) relies mainly upon myeloid MyD88, whereas that to *Pseudomonas aeruginosa* (which in contrast to *Klebsiella* potently activates TLR-5) is dependent upon epithelial cell MyD88 [15,30]. Together, these data suggest that the relative importance of myeloid and epithelial cell MyD88 for the induction of innate immune responses in the airways is dependent upon the interaction between ligands and the cell-specific expression of different MyD88-dependent receptors.

Our study has limitations. We were not able to reveal which MyD88-expressing cells are responsible for initiation

of an inflammatory response during allergic asthma. Besides bronchial epithelial cells, other cells such as mast cells and DCs have been implicated in the pathogenesis of asthma and may mediate MyD88-dependent effects in this model [27,31]. We did not cross *LysMcre-MyD88-lox* and *Sftpc^{cre}-MyD88-lox* mice, which could have provided insight into a possible interplay between myeloid and epithelial cell MyD88 in the response to HDM. In our studies we focused upon hallmark features of asthma, such as eosinophilic inflammation and mucus formation. An earlier study demonstrated a clear role for MyD88 in induction of Th2 cytokine and interleukin (IL)-17 cytokine responses [11]. In our model we were unable to confirm this finding due to non-significant increases in lung cytokine levels upon HDM administration in wild-type mice (data not shown), due most probably to the relatively long interval between the final HDM challenge and organ harvesting for analyses (2 days). Also, total serum IgE levels were high in MyD88-deficient mice before being challenged with HDM (data not shown), consistent with previous reports [11], which makes this readout less suitable to use in these animals. Furthermore, we did not study the effect of cell-specific MyD88 deficiency on airway resistance, reported previously to be abrogated in full MyD88KO mice [11].

Understanding the exact mechanisms responsible for the development of chronic airway diseases such as asthma should enable the development of more cell-targeted therapeutics for patients. The present study confirms a previous report on the pivotal role for MyD88 in allergic airway inflammation induced by the common human allergen HDM in mice [11], but by making use of cell-specific tissue KO mice for the first time demonstrates that macrophages and type II epithelial cells are not involved in this MyD88-dependent effect. Further investigations are warranted to dissect in which cell type MyD88 plays a critical role in HDM-induced lung inflammation.

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Author contributions

A. A. A. conceived and carried out experiments. A. A. A., J. Y., J. D. de B., A. F. de V. and T. v. d. P. conceived and analysed data. J. T. H. R. and B. H. analysed the data. All authors were involved in writing the paper and had final approval of the submitted and published versions.

Disclosure

The authors declare no commercial or other associations that might pose a conflict of interest.

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Supporting information

Additional Supporting information may be found in the online version of this article at the publisher's web-site:

Table S1. Cre-mediated deletion of myeloid differentiation factor 88 (MyD88)

SUPPLEMENTARY TABLE 1. Cre-mediated deletion of MyD88.

	LysMcre-MyD88-lox	Sftpcrc-MyD88-lox
Cell population	Percentage of MyD88 deletion	Percentage of MyD88 deletion
Macrophages	83.5	0*
Splenocytes	9.6	11.7
Lung epithelial cells	19.1	82.7

DNA of primary cells derived from LysMcre-MyD88-lox and Sftpcrc-MyD88-lox mice and their respective controls (n = 4 per group) was isolated to calculate cre-mediated deletion efficiency of MyD88. This was done by quantifying the residual amount of *MyD88^{fl/fl}* allele via qPCR relative to the unaffected Socs-3 gene. The amount of remaining “floxed” MyD88 region in LysMcre-MyD88-lox and Sftpcrc-MyD88-lox mice was calculated using the 2^{-deltaCt} ($\Delta\Delta C_t$) method, and using the amount of genomic DNA from the corresponding control mice for the no-deletion control. The percentage of MyD88 deletion was then calculated as $(1 - \text{residual } MyD88^{fl/fl}) \times 100$. The percentages of MyD88 deletion in LysMcre-MyD88-lox presented here were adapted from data previously reported by our group [1]. *The value “0” here represents a calculated number of percentage deletion that was negative.

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