

Research paper

MyD88 modulates eosinophil and neutrophil recruitment as well as IL-17A production during allergic inflammation

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ABSTRACT

The contribution of dysregulated innate immune responses to the pathogenesis of allergic disease remains largely unknown. Herein, we addressed the role of Toll-like receptor signaling in airway inflammation by studying mice rendered deficient for the myeloid differentiation factor 88 (MyD88^{-/-}) which results in concurrent deficiencies in TLR and IL-1R1 signaling pathways. We show that the lack of MyD88 offers a partial protection from allergic disease evidenced by reduced airway eosinophilia and production of the Th17-associated effector cytokine IL-17A. By contrast, airway hyperreactivity and Th2 cytokine production, the cardinal features of allergic disease, remained unchanged. We found that the impaired IL-17A production in MyD88^{-/-} mice was associated with defective CD4⁺ T cells, which failed to respond to IL-23 stimulation. The total number of Th17-associated effectors in lymph nodes was likewise decreased. Taken together, our results demonstrate that MyD88-dependent mechanisms are critical for orchestrating lung inflammatory responses, in terms of IL-17A production, as well as eosinophil and neutrophil recruitment.

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1. Introduction

The myeloid differentiation factor 88 (MyD88) is the key adaptor protein for the interleukin-1 (IL-1) receptor and most Toll-like receptors (TLRs), which are required for innate immunity and pathogen-associated molecular pattern recognition. For this reason, MyD88-deficient mice are more susceptible to many pathogens [1]. However, studies of various pathologies in MyD88-deficient mice have revealed a number of anomalies, which remain poorly understood. MyD88 has initially been described as a gene important for terminal differentiation of myeloid cells induced by IL-6 [2]. Later on, its role in early hematopoiesis has been established since both hematopoietic stem cells (HSCs) and some lymphoid cells require MyD88-dependent activation for their differentiation [3,4].

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Asthma is a chronic inflammatory disorder of the airways clinically characterized by recurrent airflow obstruction, wheezing and airway hyperresponsiveness (AHR) [5]. It is conventionally considered a Th2-driven disease associated with airway eosinophilia, and mucus metaplasia. Yet, eosinophilic inflammation occurs in only 50% of asthmatics. Severe allergic asthma is associated with elevated levels of several mediators, including IL-6 and IL-17 [6]. The latter is increased in the lungs, sputum, bronchoalveolar lavage fluids (BALF) or sera, and correlates with the severity of airway hypersensitivity in allergic patients [7,8]. IL-17A levels are not only elevated in asthma but also in other obstructive airway diseases characterized by increased neutrophil recruitment.

It is increasingly acknowledged that the type of Th response induced is determined not only by the nature of the pathogen but also by environmental cues, including the characteristics of the tissue, and the presence or absence of inflammatory signals [9]. Th17 polarization is specifically mediated by the DC-derived cytokines IL-23 and IL-6 under the influence of transforming growth factor-β (TGF-β) [10]. It is closely associated with a range

Abbreviations: AHR, airway hyperreactivity; BALF, bronchoalveolar lavage fluid; mDC, myeloid dendritic cell; MyD88, myeloid differentiation factor 88; NOD, non-obese diabetic; OVA, ovalbumin; PBS, phosphate-buffered saline; Penh, enhanced pause; TLR, Toll-like receptor.

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of inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, inflammatory bowel diseases and bronchial asthma. In murine models of allergic airway disease the main sources of IL-17 have been shown to be Th17 cells, gamma delta T cells or macrophages [11,12]. Upon sensitization to aeroantigens, mice develop a mixed Th2/Th17 phenotype with Th2 and Th17 lymphocytes as the main actors of eosinophilic and neutrophilic forms of asthma, respectively. IL-4, IL-25, IL-33, TSLP, and Jagged-1 are important mediators of Th2-cell differentiation, while IL-1 β , IL-6, IL-21, and IL-23 are involved in the development of Th17 cells [9]. Depending on the environment, the immune responses to a pathogen may exacerbate allergic inflammation including asthma. It is also of note that, paradoxically, TLR signaling may be protective inducing immunoregulatory pathways.

Here, we examined how the lack of MyD88 affected disease outcome in a murine experimental allergic asthma model with low doses of ovalbumin (OVA). In pathogen-free conditions, the AHR was similar in MyD88^{-/-} and MyD88^{+/+} mice. However, several features of the immune responses were altered in OVA-challenged MyD88-deficient NOD mice as compared with WT controls, such as eosinophil and neutrophil counts in BALF and Th17 cytokine and chemokine levels in the lungs. These modifications were further investigated in this study, leading to the conclusion that the MyD88/IL-1R1 pathway plays a direct role in the immune control of airway inflammation.

2. Materials and methods

2.1. Mice

MyD88^{-/-} mice were kindly provided by Dr. S. Akira, Japan, crossed in the non-obese diabetic (NOD) (K^d, I-A^{g7}, D^b) genetic background and bred in our animal facilities under specific pathogen-free conditions. Note that NOD MyD88 KO mice were protected from autoimmune diabetes, and glucose levels were normal throughout the whole study [13]. All experimental procedures were performed with males to exclude any potential interference with autoimmune diabetes. All animal experiments were performed according to institutional guidelines and approved by the committee on animal experimentation of (INSERM: Institut National de la Santé et de la Recherche Médicale) and by the French Ministry of Agriculture.

2.2. Asthma induction and in vivo treatment protocol

Eight to nine week-old male C57BL/6 or NOD mice were immunized by an intraperitoneal (*i.p.*) injection of 100 μ g of chicken egg OVA (Sigma-Aldrich, Saint Quentin Fallavier, France) adsorbed onto 1.6 mg aluminium hydroxide (Merck, Fontenay-sous-Bois, France) in a volume of 0.4 ml, while controls received a saline injection. Seven days later, mice were challenged on 6 consecutive days (D7, D8, D9, D10, D11, D12) with aerosolized OVA diluted in 0.9% saline solution (5% w/v) or saline alone for 20 min. Twenty-four hours after the last challenge, AHR was measured. Mice were then killed and samples were collected for further analysis.

2.3. Measurement of airway hyperreactivity

AHR was assessed 24 h after the last challenge by whole body plethysmography (EMKA Technologies, Paris, France) and expressed as a methacholine responsiveness, as previously described [14].

2.4. Collection and analyses of bronchoalveolar lavage fluids (BALFs) and lungs

Immediately after AHR measurement, mice were anesthetized by *i.p.* injection of urethane (2 g/kg body mass, Sigma Aldrich). Airways were washed with sterile phosphate buffer saline (PBS). Total and differential cell counts were determined in BALF after May-Grünwald/Giemsa (Merck) staining of cells on cytospin slides. Lungs were frozen in 1 ml of PBS and cytokines were measured in lysates [15].

2.5. Isolation of lung mononuclear cells (MNCs)

To obtain intraparenchymal lung MNC, the lung was minced, and incubated in RPMI 1640 supplemented with 5% FCS, 100 U penicillin/streptomycin, 10 mM HEPES, 50 μ M 2-ME, 20 mM L-glutamine containing 20 U/ml collagenase and 1 g/ml DNase type I. After a 60-min incubation at 37 °C, any remaining intact tissue was disrupted by passage through a 75 μ m filter to remove the major tissue fragments, and cells were collected by centrifugation. The cell pellet was suspended in 5 ml of 40% Percoll and layered onto 5 ml of 70% Percoll, centrifuged (2400 rpm, 4 °C, 30 min) and then collected.

2.6. T-cell purification

For CD4⁺ T-cell isolation, spleens and peripheral lymph nodes were mechanically disrupted through a 70 μ m cell strainer. CD4⁺ cells were isolated by positive immunoselection using CD4 (L3T4) microbeads (Miltenyi Biotec).

2.7. Cytokine and chemokine analysis

Samples were collected and cytokines (IL-4, IL-5, IL-13, IL-17) and chemokines (eotaxin-1 or CCL11, CCL2) were measured by ELISA (R&D Systems). Recombinant cytokines used were: IL-6, IL-12 and TGF- β (2 ng/ml); IL-23 (10 ng/ml) (R&D Systems).

2.8. Antibodies

The following mAbs were purchased from eBioscience: PerCp-Cy5.5 or Pacific blue-conjugated anti-CD4 (RM4-5), APC-conjugated anti-IL-17A (eBio17B7), and APC-conjugated anti-IFN- γ (XMG1.2). APC-Cy7-conjugated anti-c-kit (2B8) was purchased from SouthernBiotech). Anti-CD3 and anti-CD28 mAbs were from BD Pharmingen.

2.9. Th17 polarization

Th17 differentiation was induced *in vitro*. Briefly, purified CD4⁺ T cells (2×10^5 /well) cultured with IL-2 (100 IU/ml) were stimulated with plate bound anti-CD3 (1 μ g/ml) and anti-CD28 (2 μ g/ml) in the presence of 1 ng/ml rhTGF- β , 10 ng/ml IL-6, or 10 ng/ml IL-12 in the presence or absence of 10 ng/ml IL-23 for 72 h at 37 °C before measuring IL-17 levels in supernatants by ELISA.

2.10. Flow cytometry

Spleen and lymph node cells were dissociated by mechanical shearing in PBS containing 0.5% bovine serum and 2 mM EDTA, and then filtered through a 100- μ m mesh. The cell suspension was filtered through a 100- μ m mesh. Dead cells and debris were removed over a Percoll gradient. The preparation was enriched using autoMACS (after incubation with biotinylated antibodies specific) and anti-biotin beads (Miltenyi Biotec). For surface staining, cells were first incubated with 10% FCS containing DME at

37 °C for 30 min in the presence of antibody, followed by staining at 4 °C for other surface markers. Samples were collected and measured on a FACS Cantoll cytometer (BD Biosciences). Mononuclear cells were gated according to forward- and side-scatter characteristics using the FACS Diva software, and analyzed with Flowjo software (Tree Star).

2.11. Intracellular cytokine staining

For intracellular staining of cytokines, cells suspended in DME were stimulated for 3 h with 50 ng/ml PMA and 500 ng/ml ionomycin (Sigma-Aldrich). For the last 1.5 h, 10 µg per ml brefeldin A (Sigma-Aldrich) was added to the culture medium. After cell surface staining for CD4, cells were fixed with 2% PFA (Sigma-Aldrich) and permeabilized with 0.5% saponin (Sigma-Aldrich). Intracellular staining was performed using antibodies against IL-17A and IFN-γ.

2.12. Cell culture

mDCs were obtained from bone marrow-derived cells cultured in the presence of GM-CSF for 8 days as previously described [16]. Sorted CD4⁺ T cells were cultured in RPMI 1640 culture medium (Invitrogen) containing 10% FCS, β2-mercaptoethanol, penicillin, and streptomycin, and seeded into flat-bottom 96-well plates in the presence of 10 ng/ml mouse rIL-12, rIL-23, TGF-β and IL-6. In some experiments, plates were coated with anti-CD3 and anti-CD28 mAbs. Alternatively, cells were incubated with mDCs and CD4⁺ T cells at a ratio 1:5. After a 3-d culture, cells were recovered as described above and analyzed by flow cytometry.

2.13. Statistical analysis

Data from *in vivo* experiments are presented as means ± SEM, while data from *in vitro* experiments (CD4⁺ T cell/BMDC cocultures) are presented as means ± SD. Data were analyzed by ANOVA or unpaired Student's *t* test using Prism 4 (GraphPad Software Inc., La Jolla, CA). Significance for all statistical tests is indicated in figures as (*) for *p* < 0.05, (**) for *p* < 0.01 and (***) for *p* < 0.001.

3. Results

3.1. MyD88 signaling is required for optimal development of OVA-induced allergic inflammation

To investigate the role of MyD88 signaling in the development of allergic asthma, we used the OVA-induced model in NOD mice, which have previously been identified as effective responders in this experimental setup [17]. Asthmatic responses were induced by systemic immunization with ovalbumin (OVA, 100 µg, i.p.) precipitated in aluminium hydroxide (alum) as adjuvant, followed by multiple respiratory tract challenges (20 min each) by OVA in aerosol solution (50 mg/ml). As shown in Fig. 1A, the lack of MyD88 had no significant effect on OVA-induced AHR measured by Penh as compared with WT mice.

Untreated MyD88-deficient and WT mice shared a similar baseline cell count in BALF (Fig. 1B). However, in response to OVA challenge, total cell (data not shown), eosinophil and neutrophil recruitment were markedly reduced in mice lacking MyD88, conversely to macrophages and lymphocytes.

In lung homogenates the levels of Th2 cytokines, namely IL-4, IL-5 and IL-13, were increased after OVA challenge in both WT and MyD88^{-/-} mice, relative to their saline controls, as expected (Fig. 1C). There was no significant difference between the two strains. By contrast, IL-17 concentrations were strikingly reduced

in MyD88^{-/-} mice (Fig. 1C) after OVA challenge, which was also the case for CCL-11 (eotaxin-1), but not for CCL2.

3.2. Impaired neutrophil infiltration in lungs from MyD88^{-/-} mice

To further assess the contribution of MyD88 to neutrophilia, we tested the cell recruitment in lungs from both strains following treatment with α-GalCer, a potent inflammatory glycolipid, which targets specifically natural killer T cells [18]. We found that airway neutrophilia induced by α-GalCer instillation was significantly reduced within 24 h in the bronchoalveolar lavage fluid of MyD88-deficient mice as compared to WT controls (Fig. 2), which proved that MyD88 signaling participates in neutrophil recruitment during airway inflammation. In the light of these results one would also expect a modification of the cytokine/chemokine profile of innate cells which mediate glycolipid-induced neutrophilia, in MyD88^{-/-} versus WT mice.

3.3. Defective Th17 response of CD4⁺ T cells from MyD88-deficient mice

To evaluate the inflammatory response in terms of Th17 differentiation, we compared the *in vitro* IL-17A production by CD4⁺ cells from NOD MyD88^{+/+} and MyD88^{-/-} mice. Sorted CD4⁺ T cells from splenocytes were stimulated with anti-CD3 and anti-CD28 mAbs in the presence of anti-IL-4 and anti-IFN-γ (inhibiting Th1 and Th2 pathways) and the cytokines IL-23, IL-12 (control of IL-23) or IL-6 with or without TGF-β. As shown in Fig. 3A IL-23, was by far the most efficient inducer of IL-17A production by WT CD4 cells, while their MyD88^{-/-} counterpart was unresponsive. Similar results were observed when CD4⁺ T cells were exposed to IL-23 in the presence of myeloid dendritic cells (mDCs) (Fig. 3B). By contrast, MyD88 deficiency did not affect IFN-γ production under Th1 cell-polarizing conditions (data not shown), which proves that antigen-specific IL-17A production depends on the expression of MyD88. Note that under Th17-polarizing conditions (TGF-β + IL-6), MyD88^{-/-} CD4⁺ T cells produced only slightly less IL-17A during 5 days of culture in the presence of DC than their WT counterpart (Fig. 3C), suggesting that their unresponsiveness was not due to reduced RORγt expression. Hence, the most pronounced impairment of IL-17A production occurred after restimulation of CD4⁺ T from MyD88^{-/-} mice, suggesting that MyD88 is required for IL-23 signaling during later Th17 differentiation stages.

3.4. Impaired regulation of Th17 cell expansion in MyD88^{-/-} mice

We addressed the role of MyD88 in promoting Th17 responses in mice maintained under pathogen free conditions. To this end, IL-17A concentrations were measured in the serum of both MyD88-deficient and WT mice, together with the cytokines that induce Th17 differentiation, IL-6 and TGF-β, we found a selective reduction of IL-17A and IL-6 levels in sera from animals lacking MyD88, but no alteration in the amount of TGF-β (Fig. 4A). We further assessed the ability of Th17 cells to expand in MyD88-deficient mice by measuring the incidence of the CD4⁺ cell population producing IL-17A in lymph nodes. After *ex vivo* stimulation with anti-CD3 mAbs, we found reduced frequencies of CD4⁺ cells producing IL-17A in MyD88^{-/-} mice when compared with MyD88^{+/+} littermates, while IFN-γ production was not modified. These results establish a selective reduction in the frequency of the steady-state Th17 cells in the absence of MyD88 (Fig. 4B).

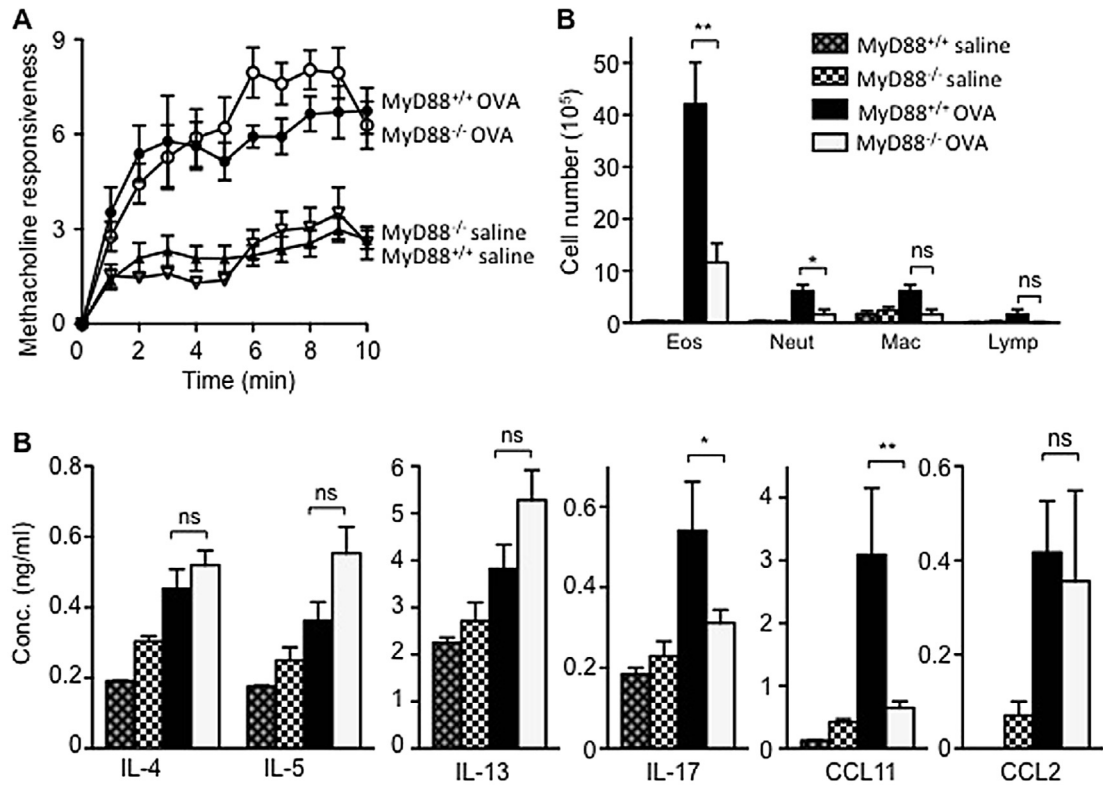


Fig. 1. OVA-induced allergic airway inflammation is dependent on MyD88. (A) Methacholine-induced whole body plethysmography in antigen-sensitized/antigen-challenged MyD88^{+/+} and MyD88^{-/-} mice. MyD88^{+/+} saline (black triangles), MyD88^{-/-} saline (white triangles), MyD88^{+/+} OVA (black circles), MyD88^{-/-} OVA (white circles). One representative experiment out of three is shown (means $\pm$ SEM, NS, two-way ANOVA, Bonferroni post-test). (B) Eosinophil (eos), neutrophil (neut), macrophage (mac) and lymphocyte (lymp) counts in BALF. MyD88^{-/-} mice compared with MyD88^{+/+} mice (MyD88^{+/+} n = 5, MyD88^{-/-} n = 5; means $\pm$ SEM, *p < 0.05, **p < 0.01, Student's *t*-test). (C) IL-4, IL-5, IL-13, IL-17, CCL11 and CCL2 levels in lung homogenates from saline- and OVA-treated MyD88^{+/+} and MyD88^{-/-} mice. (Means $\pm$ SEM, n = 5, *p < 0.05 and **p < 0.01, one-way ANOVA, Dunnett's multiple comparison).

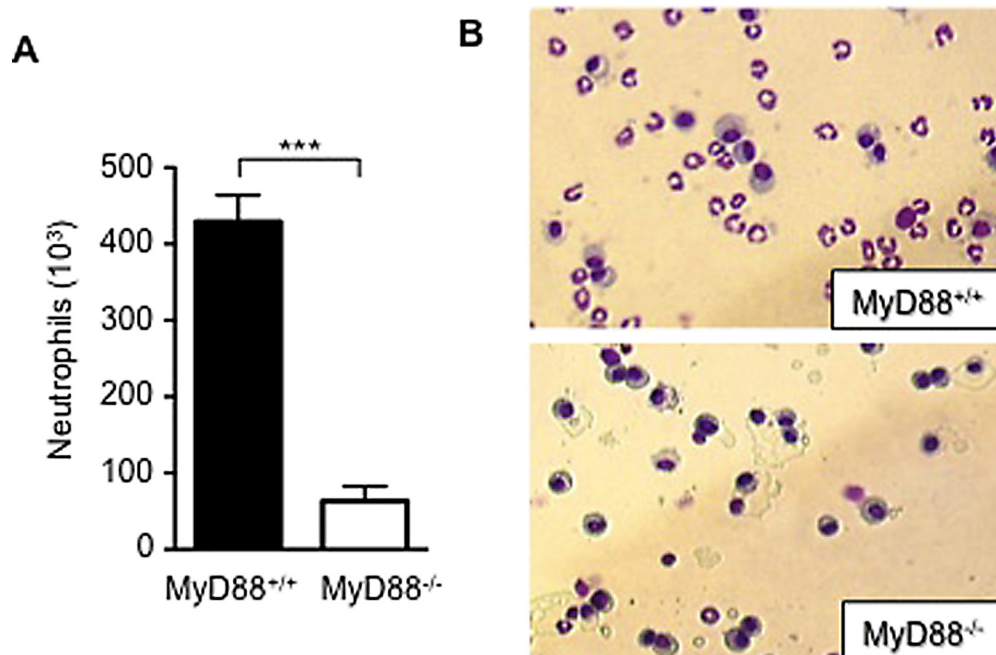


Fig. 2. Neutrophil recruitment in BALF in lung tissue induced by α -GalCer stimulation was reduced in MyD88^{-/-} mice. Total number of neutrophils in BALF from WT and MyD88^{-/-} mice following i.n. α -GalCer treatment (means $\pm$ SEM from pooled data from two independent experiments; ***p < 0.001; n = 8 mice per group).

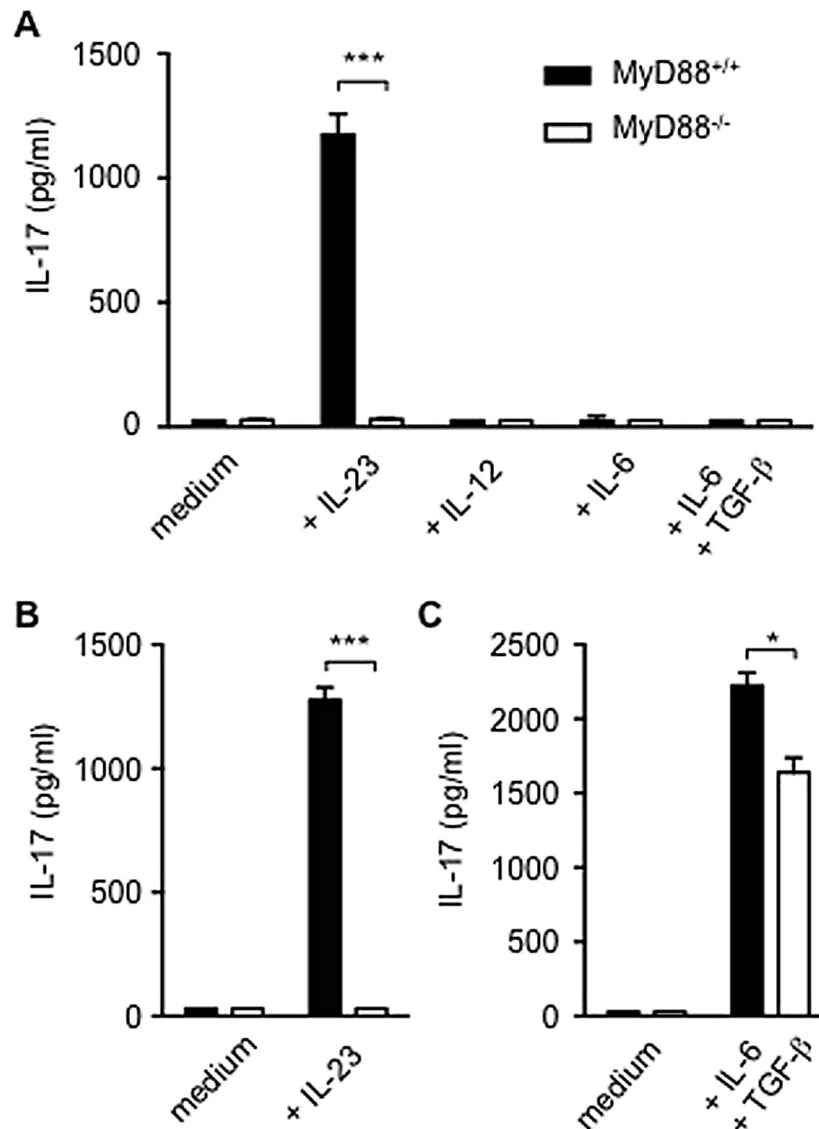


Fig. 3. Impaired IL-17A production by T cells from MyD88^{-/-} mice. (A) Sorted splenic CD4⁺ T cells from MyD88^{+/+} and MyD88^{-/-} mice were stimulated with 10 ng/ml IL-23, IL-12 or IL-6 with or without TGF-β for 72 h at 37 °C before measuring IL-17A levels in supernatants by ELISA using plate bound anti-CD3 (1 μg/ml) and anti-CD28 (2 μg/ml). (B) Sorted cells were prepared as described in panel A and stimulated using plates with uncoated wells in the presence of mDCs. Similar results were observed in 3 independent experiments. (C) Purified MyD88^{-/-} or MyD88^{+/+} CD4⁺ T cells were cultured under Th17-polarizing conditions for 5 days in the presence of IL-6 plus TGF-β and mDCs, followed by restimulation with plate-bound anti-CD3 for 24 h. ELISA was performed to detect the amounts of IL-17A in culture supernatants. (A, B, C) Data are presented as means ± SEM; * p < 0.05, *** p < 0.001, one-way ANOVA, Dunnett's multiple comparison test to medium.

4. Discussion

The critical role of the TLR/IL-1R1/MyD88 signaling pathway in infectious diseases and Th1 immune responses, prompted us to examine how it affected the induction and maintenance of allergic inflammatory responses. We found that both AHR and Th2 cytokine production remained unchanged in mice lacking MyD88. By contrast, eosinophil and neutrophil counts were diminished in their BALF, as well as IL-17 and CCL-11 levels in lungs.

OVA- and α-GalCer-induced inflammation induced decreased recruitment of eosinophils and neutrophils, respectively, in mice lacking MyD88 which is in accordance with previous findings obtained in a distinct model of lung pathogenesis, namely bleomycin-induced acute lung inflammation [19]. Interestingly, MyD88 signaling is required for inducing neutrophil chemoattractants, such as CXCL1 and CXCL2, as well as neutrophil influx. Decreased granulocyte recruitment resulting from MyD88 defi-

ciency has been observed in experimental inflammatory models other than the lung [20,21]. Conversely, our results concur with previous studies showing that Th2 priming depends on IL-4 but not on MyD88 [22,23].

MyD88 deficiency resulted in a striking decrease of Th17 production by CD4⁺ T cells, which according to a recent report behave like lung resident Th2 memory/effector cells with inflammatory properties and contribute to the exacerbation of chronic asthma [24]. Consistent with this notion, their incidence was decreased in MyD88^{-/-} mice relative to MyD88^{+/+} littermates. Our data fit also with other experimental models providing evidence for a defective Th17-mediated response with decreased producer cells in MyD88-deficient mice during *Helicobacter* infection, inflammatory bowel disease or experimental autoimmune encephalomyelitis [25–27]. Consistent with its implication in lung inflammation, MyD88 has been reported for being critically involved in immune tolerance and associated with a reduced IL-17A and IFN-γ production in

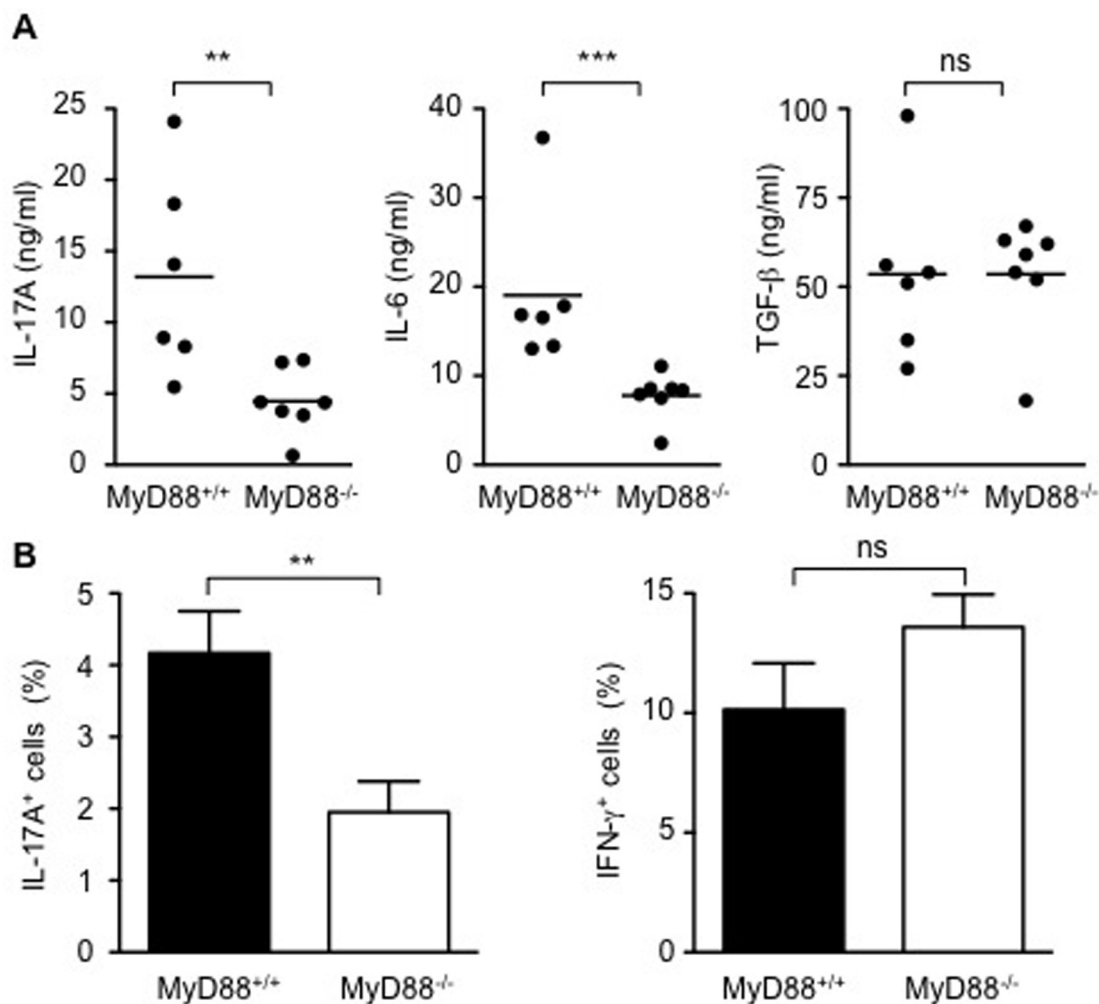


Fig. 4. MyD88-deficient mice have a reduced population of Th17 cells. (A) Decreased levels of IL-17A and IL-6 in serum of MyD88^{-/-} mice concomitant with unchanged amounts of TGF-β (MyD88^{+/+} *n* = 6, MyD88^{-/-} *n* = 7, Student's *t*-test, ***p* < 0.01, ****p* < 0.001). (B) Decreased percentage of memory CD4⁺ T cells expressing IL-17A in LN from MyD88^{-/-} versus MyD88^{+/+} mice, in contrast with unchanged IFN-γ production (*n* = 8–12 mice per group, ***p* < 0.01, Student's *t*-test).

Foxp3-deficient mice [28]. The unchanged IFN-γ production in our model may be explained by a stronger inflammation resulting from Foxp3-deficiency. Taken together, our observations validate the notion that the homeostatic development of Th17 cells requires MyD88 signaling.

Even though eosinophils are the most prominent cells at sites of allergic inflammation, the decreased airway eosinophil infiltration in MyD88^{-/-} mice illustrates that they are dispensable for the development of allergic asthma and AHR. In the same line of evidence, a number of studies have reported that airway eosinophilia and hyperresponsiveness after allergic reaction are mediated by distinct mechanisms [29,30], a notion that was further confirmed in mice selectively depleted of eosinophils [31].

Among Th2 cytokines modulating eosinophilia, IL-5 is considered most important for eosinophil infiltration of the airways [32]. However, in our study, the IL-5 levels in BALF did not differ significantly between WT and MyD88^{-/-} mice before and after antigen challenge. Therefore, IL-5 is not likely to be involved in the decreased eosinophil accumulation in the airways of MyD88^{-/-} mice.

MyD88 is a common adaptor molecule of TLR and IL-1R families, localized in the cytoplasmic region of a conserved sequence called Toll/IL-1R (TIR) domain. Members of the IL-1 family have been described as activators and suppressors of allergic inflamma-

tion. Indeed, IL-1β, which is promptly produced during inflammatory responses, may be significantly involved in the development of pulmonary Th2 responses and the etiology of bronchial asthma by inducing the expression of the potent eosinophil chemoattractant CCL-11 in pulmonary epithelial cells, and promoting recruitment of eosinophils. Consequently, pulmonary eosinophilia is strongly reduced in IL-1R1^{-/-} mice [33]. This mechanism might account for the decreased eosinophilia in our experimental setup, consecutive to a decreased rate of eotaxin production following the disruption of the IL-1 signaling in the absence of the MyD88 adaptor molecule. Another study has revealed IL-6 as a potential mediator of MyD88-dependent eosinophil recruitment [34]. Our data demonstrate that, during lung inflammation, MyD88 signaling is not only required for innate cell recruitment but also for IL-17A-producing cells. As one of the effector cytokines among the TGF-β, IL-6, IL-21 and IL-23 mixture required for CD4⁺ Th17 differentiation, IL-6 provides a powerful amplification loop in lung pathogenesis [35]. From our data, it can be proposed that the low plasma level of IL-6 measured in MyD88-deficient mice is associated with its failure to promote the Th17 differentiation process. It has been shown that MyD88^{-/-} CD4⁺ T cells have no global defect in mTOR activation [36]. In agreement with this evidence, our findings indicate that MyD88 plays a critical role in providing the adequate molecular environment leading to Th17 cell proliferation and

expansion in response to IL-23 signaling. The importance of IL-1 and IL-1R1 signaling in Th17 and autoimmune T cell activation has been extensively described [37–40]. The IL-1 receptor 1 is critical for Th2 cell type airway immune responses in a mild but not in a more severe asthma model [33]. Our data on MyD88^{−/−} fits completely with these data and we may conclude that the Th17 deficiency is derived from the cumulative effects of IL-1 α and IL-1 β deficiency. In conclusion, our findings show that MyD88 has a critical role in allergic inflammation through the IL-1R1 pathway and Th17 cell differentiation.

We hypothesize that the MyD88 pathway might be even more critical following infection, since the pathogen will not be successfully eliminated due to the lower chemokine/cytokine inflammatory response and subsequently lower innate cell infiltration. Designing curative therapies for chronic allergic diseases may require not only administration of therapeutic molecules regulating the development of various cell subsets but also the understanding of the factors that regulate the balance of immune responses and their potential interactions with the induction of allergic asthma.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

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