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Endogenous Calcitriol Synthesis Controls the Humoral IgE Response in Mice

Juliane Lindner,* Sebastian Rausch,[†] Sandra Treptow,* Kerstin Geldmeyer-Hilt,* Tina Krause,* René St-Arnaud,[‡] Alice Arabian,[‡] Andreas Radbruch,[§] Susanne Hartmann,[†] Margitta Worm,*¹ and Guido Heine*,¹

The vitamin D receptor participates in the control of IgE class-switch recombination in B cells. The physiologic vitamin D receptor agonist, 1,25(OH)₂D₃ (calcitriol), is synthesized by the essential enzyme 25-hydroxyvitamin D₃-1 α -hydroxylase (CYP27B1), which can be expressed by activated immune cells. The role of endogenous calcitriol synthesis for the regulation of IgE has not been proven. In this study, we investigated IgE-responses in *Cyp27b1*-knockout (KO) mice following sensitization to OVA or intestinal infection with *Heligmosomoides polygyrus*. Specific Igs and plasmablasts were determined by ELISA and ELISpot, *Cyp27b1* expression was measured by quantitative PCR. The data show elevated specific IgE and IgG1 concentrations in the blood of OVA-sensitized *Cyp27b1*-KO mice compared with wild-type littermates (+898 and +219%). Accordingly, more OVA-specific IgG1-secreting cells are present in spleen and fewer in the bone marrow of *Cyp27b1*-KO mice. Ag-specific mechanisms are suggested as the leucopoiesis is in general unchanged and activated murine B and T lymphocytes express *Cyp27b1*. Accordingly, elevated specific IgE concentrations in the blood of sensitized T cell-specific *Cyp27b1*-KO mice support a lymphocyte-driven mechanism. In an independent IgE-inducing model, i.e., intestinal infection with *H. polygyrus*, we validated the increase of total and specific IgE concentrations of *Cyp27b1*-KO compared with wild-type mice, but not those of IgG1 or IgA. We conclude that endogenous calcitriol has an impact on the regulation of IgE in vivo. Our data provide genetic evidence supporting previous preclinical and clinical findings and suggest that vitamin D deficiency not only promotes bone diseases but also type I sensitization. *The Journal of Immunology*, 2017, 199: 000–000.

Vitamin D mediates immunological functions via the nuclear vitamin D receptor (VDR), which is expressed in most immune cells constitutively or is activation induced (1–4). We have previously shown that activation of the VDR inhibits IgE class-switch recombination in human B cells by recruiting a transrepressive complex to the ϵ -germline gene promoter (4, 5). We confirmed this finding in vivo by analyzing a synthetic low-calcemic VDR agonist in sensitized BALB/c mice (6). Accordingly, IgE is increased in VDR-knockout (KO) mice (7). However, treatment with chem. 1,25(OH)₂D₃ (calcitriol) is limited

by hypercalcemic toxicity and synthetic VDR agonists did not reach the clinical use. Recent data support that endogenous calcitriol synthesis may target IgE in vivo. Activated human T cells and, as we have previously shown, human B lymphocytes can express the 25-hydroxyvitamin D₃-1 α -hydroxylase (CYP27B1), which catalyzes the endogenous calcitriol synthesis from its inactive precursor 25-hydroxyvitamin D₃ (1, 8–10). In addition, some epidemiologic studies support that endogenous calcitriol controls IgE because vitamin D deficiency was associated with increased serum IgE concentrations (11, 12). However, the data are heterogeneous, suggesting that other, so far unknown, cofactors are required. In a controlled preclinical setting, we have recently shown that endogenous calcitriol synthesis following repeated Ag-stimuli inhibits IgE expression (13).

In this study, we aimed to prove that endogenous calcitriol contributes to IgE regulation. Therefore, we analyzed mice deficient in endogenous calcitriol through targeted inactivation of the *Cyp27b1* gene (*Cyp27b1*-KO) regarding the IgE response upon OVA-sensitization and IgE-inducing infection with the intestinal nematode *Heligmosomoides polygyrus*.

Materials and Methods

Animal studies

All procedures were in accordance with the local State Office of Health and Social Affairs (LAGeSo, Berlin). *Cyp27b1*-KO mice (14) and wild-type C57BL/6 littermates were maintained in a specific pathogen-free environment and fed a vitamin D-deficient, rickets-preventing diet (15) containing 2% calcium, 1.25% phosphorus, and 20% lactose (Sniff, Germany or Altromin, Germany) or regular chow containing 1000 kU vitamin D₃ per kg, respectively.

Conditional *Cyp27b1*-KO mice were generated by crossing *Cyp27b1*^{fl/fl} mice (14) with mice expressing Cre-recombinase under transcriptional control of B cell-specific *Mb1* promoter (16) or T cell-specific *Cd4* promoter (17), respectively.

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Abbreviations used in this article: ASC, Ab-secreting cell; calcitriol, 1,25(OH)₂D₃; CYP27B1, 25-hydroxyvitamin D₃-1 α -hydroxylase; KO, knockout; VDR, vitamin D receptor.

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Analysis of the leucopoiesis

The analysis of the leucopoiesis of vitamin D-deficient *Cyp27b1*-KO and wild-type mice was carried out following standard protocols (18).

OVA sensitization

Female adult mice (10–14 wk) were sensitized as described previously (19) on days 0, 14, and 21 using 10 μ g OVA (Sigma, Germany) adsorbed to 1.5 mg aluminum hydroxide and magnesium hydroxide (Imject Alum; Thermo Fisher Scientific, Germany) in 100 μ l 0.9% NaCl injected i.p. Mice were challenged on day 50 by 10 μ g OVA in 100 μ l 0.9% NaCl i.p. and day 58 and 99 using 100 μ g OVA in 20 μ l 0.9% NaCl s.c. as depicted in Fig. 1A. Blood samples were collected on days 0, 35, 56, and 100.

Parasite infection with the intestinal nematode *H. polygyrus*

H. polygyrus was maintained by passage in C57BL/6 mice. The primary infection was performed in adult *Cyp27b1*-KO mice and wild-type littermates by applying 200 L3 larvae orally (Fig. 5A). On day 14 mice received a single dose of pyrantel pamoate (0.5 mg per mouse; Sigma) via oral gavage to eliminate parasites. On day 76 mice were challenged with 200 L3 larvae. Blood samples were collected on days 0, 13, 76, and 90.

Ig measurement

The Ig concentrations in the blood were determined from plasma by ELISA as described previously regarding OVA-specific IgE (19, 20) and IgG1 (6). OVA-specific IgA was detected using 0.5 μ g/ml biotinylated anti-mouse IgA (Southern Biotech, Germany). Total IgE concentrations were determined as described previously (6) and total IgG1 and total IgA were determined using matched isotype-specific alkaline phosphatase-coupled detection Abs (all Southern Biotech). Total and OVA-IgG2c Abs were detected using a Mouse IgG2c ELISA Quantitation Set (Bethyl Laboratories) according to the manufacturer's protocols. Ig concentrations were determined using a standard curve obtained from diluted pooled plasma of OVA-sensitized mice or monoclonal Ig-isotypes followed by four-point curve calculations. Data are expressed as laboratory units per milliliter or as international system of units, respectively.

The concentrations of *H. polygyrus*-specific Abs were determined by ELISA from titrated plasma incubated on Nunc MaxiSorp plates coated with isotype-specific Abs diluted in PBS (5 μ g/ml anti-IgE, clone R35-72; BD Biosciences; 0.5 μ g/ml anti-IgG1, 2.5 μ g/ml anti-IgA; both Southern Biotech). After binding of biotinylated excretory-secretory products of *H. polygyrus* (21), the streptavidin-HRP (R&D Systems, Germany)-driven colorimetric reaction of the peroxidase substrate tetramethylbenzidine (Sigma) was quantified and calculated as described above. The frequencies of Ab-secreting cells (ASC) were determined by ELISpot, as described (22).

Calcitriol-ELISA

The concentrations of calcitriol in the plasma of 5–21 mo old *Cyp27b1*-KO mice and wild-type littermates were determined commercially (Immunodiagnostik, Germany) using a competitive enzyme immunoassay following solid phase extraction of calcitriol from the vitamin D binding protein.

RNA extraction, cDNA synthesis, and quantitative real-time PCR

For the analysis of *Cyp27b1* mRNA expression, purified CD19⁺ B cells and CD4⁺ T cells from C57BL/6-mice were stimulated for 24 h with 10 μ g/ml anti-CD40 (clone FGK-45, DRFZ, Berlin) and 20 ng/ml IL-4 (R&D Systems) or 3 μ g/ml anti-CD3 (clone 145-2C11; Miltenyi Biotec, Germany) and 1 μ g/ml anti-CD28 (clone 37.51; DRFZ, Berlin), respectively. RNA extraction (Macherey Nagel, Germany), reverse transcription (Applied Biosystems, Germany), and quantitative PCR (SYBRgreen PCR Kit on Rotor-Gene Q; Qiagen, Germany) were performed according to the manufacturers' protocols. All oligonucleotides were synthesized by TIB MOLBIOL (Berlin, Germany) and are given in 5'-3' format: *Hprt* 5'-cgt cgt gat tag cga tga tg-3' (forward), 5'-aat cca gca ggt cat caa ag-3' (reverse); *Cyp27b1* 5'-ctt tgg gac act tcg cac ag-3' (forward), 5'-ctt egg agc ctc tgc cat t-3' (reverse). The melting curve analysis was used to control the melting point specificity. Efficiency-corrected relative quantification was performed by the $2^{-\Delta\Delta}$ cycle threshold method.

Statistical analysis

For determination of statistical significance, datasets were analyzed using GraphPad Prism, version 6.0 (GraphPad Software). Normal distribution was tested using a Kolmogorov–Smirnov test. Data are shown accordingly as

single values and median or mean $\pm$ SEM and analyzed using Mann–Whitney *U* test or *t* tests. A *p* value < 0.05 was considered statistically significant.

Results

Increased humoral IgE immune response in *Cyp27b1*-KO mice

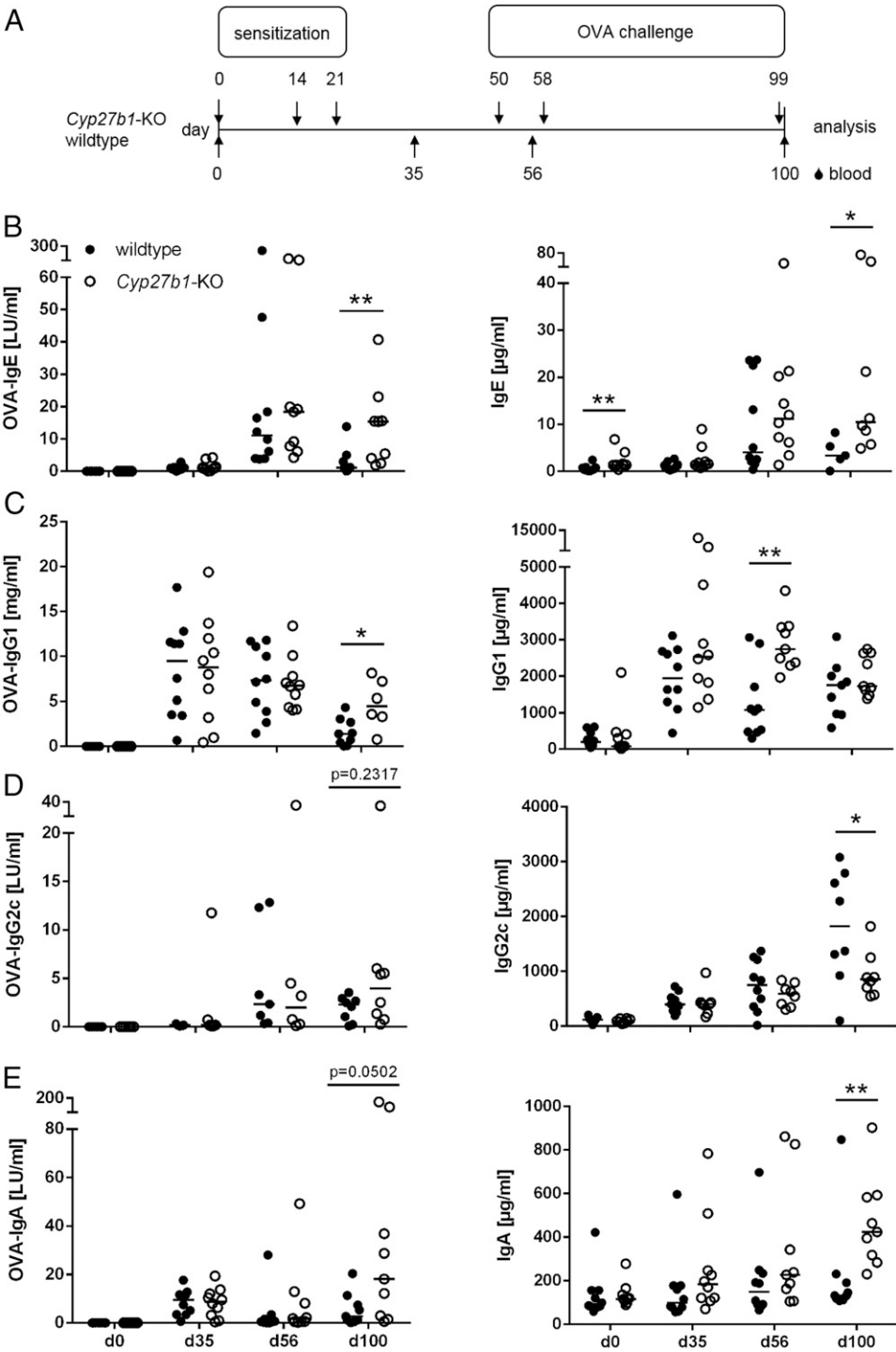
We have shown previously that activated immune cells express the enzyme CYP27B1, and in BALB/c mice with or without restriction in nutritional vitamin D, endogenous calcitriol inhibits IgE induction (13). To prove this finding at the genetic level, we investigated *Cyp27b1*-KO mice, lacking exon 8 containing the essential heme-binding domain (14). We confirmed the knockout of the *Cyp27b1* gene by measuring plasma concentrations of calcitriol, proving calcitriol deficiency of *Cyp27b1*-KO mice (Supplemental Fig. 1).

Next, *Cyp27b1*-KO mice and wild-type littermates were sensitized to OVA and the humoral Ig response was measured over time (Fig. 1A). The data show an induction of OVA-specific isotypes IgE, IgG1, IgG2c, and IgA in a comparable manner between both genotypes following sensitization until day 56 (Fig. 1B–E). Then, after OVA challenge, *Cyp27b1*-KO mice showed increased OVA-specific Ab concentrations at day 100 compared with wild-type mice. In detail, OVA-IgE and OVA-IgG1 increased significantly (10-fold and 3.2-fold, respectively), whereas OVA-IgG2c and OVA-IgA increased by trend (1.7-fold and 6.7-fold). Similarly, the total Ig classes did not differ between the genotypes before OVA challenge with the exception of slightly elevated total IgE levels in *Cyp27b1*-KO mice before immunization. After challenge, *Cyp27b1*-KO mice displayed significantly increased levels of total IgE (3.1-fold), IgG1 (2.5-fold), and IgA (3.2-fold), whereas IgG2c was decreased (–2.1-fold) (Fig. 1B–E).

Next, we investigated whether endogenous calcitriol acts on the humoral immune response by reducing the numbers of OVA-specific ASCs or by altered cell trafficking applying ELISpot analysis, as the localization of ASC can impact their lifespan, e.g., plasmablasts reside for days in inflamed or lymphoid tissue and plasma cells can populate the bone marrow lifelong (23). In initial pilot experiments, sensitization-induced OVA-IgE-secreting cell numbers were at the detection threshold (data not shown) as described earlier (24). Thus, we focused on IgG1-secreting cells, because both are induced by the same stimuli (25) serving as surrogate marker for IgE Ab responses. The data show tissue-specific differences with increased frequencies of OVA-specific IgG1-secreting cells in the spleen of *Cyp27b1*-KO mice compared with wild-type littermates, whereas frequencies of OVA-IgA-secreting B cells were similar (Fig. 2). In the bone marrow, OVA-IgG1 and also OVA-IgA ASCs were decreased 2.1-fold and 2.4-fold, suggesting a reduced homing and/or survival of these cells in the bone marrow compartment in *Cyp27b1*-KO mice (Fig. 2).

Ag-specific mechanisms in the humoral immune response in calcitriol-deficient mice

To further determine whether Ag-independent adaptive immune mechanisms such as constitutional depletion of a defined cell lineage or Ag-dependent mechanisms mediate the altered OVA-specific response in *Cyp27b1*-KO mice, we analyzed the leucopoiesis in adult mice and *Cyp27b1* expression in B and T lymphocytes. Confirming previous data, adult mice of both genotypes present a comparable body weight, following a slightly reduced weight of young *Cyp27b1*-KO mice until a mean age of 8 wk (data not shown). At an age of 24 wk, trends for reduced thymic (–80.3%) and splenic (–57.3%) cell numbers were detected, but bone marrow and the peritoneal exudate cellularity



were comparable between both genotypes (data not shown). Flow cytometric analysis showed the presence of all major cell types including B cells (B-1 cells, B-2 cells, immature B cells, mature

B cells), T cell subpopulations, and myeloid cell types. Of note, in the bone marrow subtle different frequencies were suggested regarding fewer pre-B cells, immature B cells, switched B cells, and

FIGURE 2. Reduced number of ASC in bone marrow of *Cyp27b1*-KO mice. Mice deficient in endogenous calcitriol (*Cyp27b1*-KO) and wild-type littermates were treated as described in Fig. 1. The numbers of ASC were analyzed from spleen and bone marrow on day 100 regarding (A) OVA-IgG1 and (B) OVA-IgA by ELISpot. The lines indicate the median of $n = 7-9$ mice. * $p \leq 0.05$, ** $p \leq 0.01$.

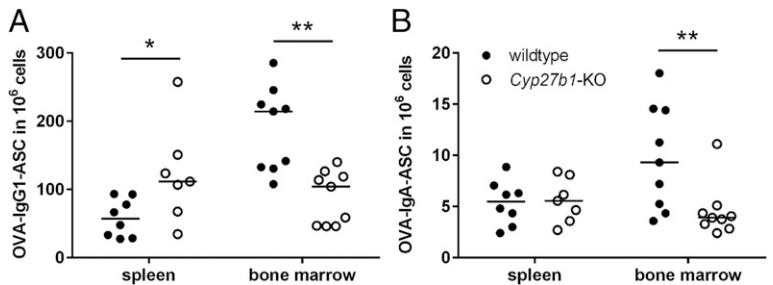
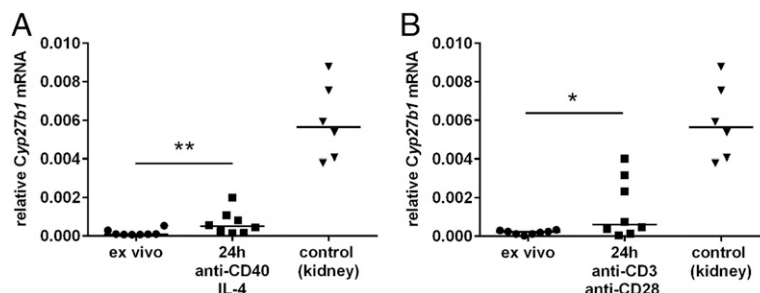


FIGURE 3. Stimulated B and T cells express *Cyp27b1* mRNA. Murine splenocytes were purified into (A) CD19⁺ B cells and (B) CD4⁺ Th cells and cultured in the presence of anti-CD40 and IL-4 or anti-CD3 and anti-CD28, respectively. The transcription of the vitamin D-metabolizing gene *Cyp27b1* was determined by quantitative PCR and is shown relative to *Hprt* expression. The lines indicate the median of $n = 6$ –8 mice. * $p \leq 0.05$, ** $p \leq 0.01$.



CD11b^{int} Gr-1^{high} myeloid cells in *Cyp27b1*-KO mice (Supplemental Tables I–III).

Next, we determined the *Cyp27b1* expression in murine B and T lymphocytes, which are crucial for the humoral OVA responses, and which is activation induced in human lymphocytes (8, 10). The data show low *Cyp27b1* mRNA expression in resting B and T lymphocytes (Fig. 3). Upon stimulation with anti-CD40 and IL-4, or anti-CD3 and anti-CD28, splenic murine CD19⁺ B (Fig. 3A) and CD4⁺ T cells (Fig. 3B) showed transiently enhanced expression of *Cyp27b1* after 24 h (5.2-fold and 2.8-fold). These data confirm in mice earlier findings shown in humans.

Increased IgE response in T cell-specific *Cyp27b1*-KO mice

To dissect whether the observed Ig regulation results from lymphocyte or stromal (e.g., kidney) cell *Cyp27b1* expression, the B or T lymphocyte-specific gene function was deleted by crossing mice expressing *Mb1*- or *Cd4* promoter-driven Cre-recombinase to *Cyp27b1* exon 8-floxed mice. The data show significantly increased plasma OVA-IgE on day 56 and OVA-IgE-secreting cells in spleen and bone marrow of T cell-specific *Cyp27b1*-KO mice compared with controls (Fig. 4). In B cell-specific *Cyp27b1*-KO mice, no significant differences in specific Ig-plasma concentrations were determined, however, by trend more IgE-secreting cells were observed in the spleen compared with control. The plasma concentrations and numbers of OVA-specific IgG1-secreting cells show no significant differences between the genotypes (data not shown).

Increased IgE response in *Cyp27b1*-KO mice upon helminth infection

To prove whether the regulation of the humoral IgE response by endogenous calcitriol is restricted to the applied immunization and Ag recall setting or also valid in an infection model, we analyzed *Cyp27b1*-KO and wild-type mice during the strongly IgE-inducing infection with the gastrointestinal nematode *H. polygyrus*. Therefore, adult mice were infected orally with *H. polygyrus*, the primary infection was abrogated after 2 wk and the immunological memory response was challenged by a secondary infection on day 76, which was determined at the humoral level on day 90 (Fig. 5A). Confirming our data from OVA-sensitized mice *Cyp27b1*-KO mice showed elevated levels of *H. polygyrus*-specific (1.4-fold) and total (1.5-fold) IgE after challenge infection (Fig. 5B). The plasma concentrations of *H. polygyrus*-specific IgG1 or IgA showed no differences between the genotypes, as well as total IgG1, and total IgA was marginally altered (Fig. 5C, 5D).

Discussion

This study shows that mice lacking endogenous calcitriol due to targeted inactivation of the *Cyp27b1* gene exhibit an enhanced humoral IgE and IgG1 response following OVA sensitization. This function is accompanied by increased numbers of specific ASCs in the spleen. We identified *Cyp27b1* mRNA in activated murine B and T lymphocytes and a regular leucopoiesis in calcitriol-deficient mice, suggesting a specific function of endogenous calcitriol during the Ag-specific immune reaction. Applying an independent IgE-

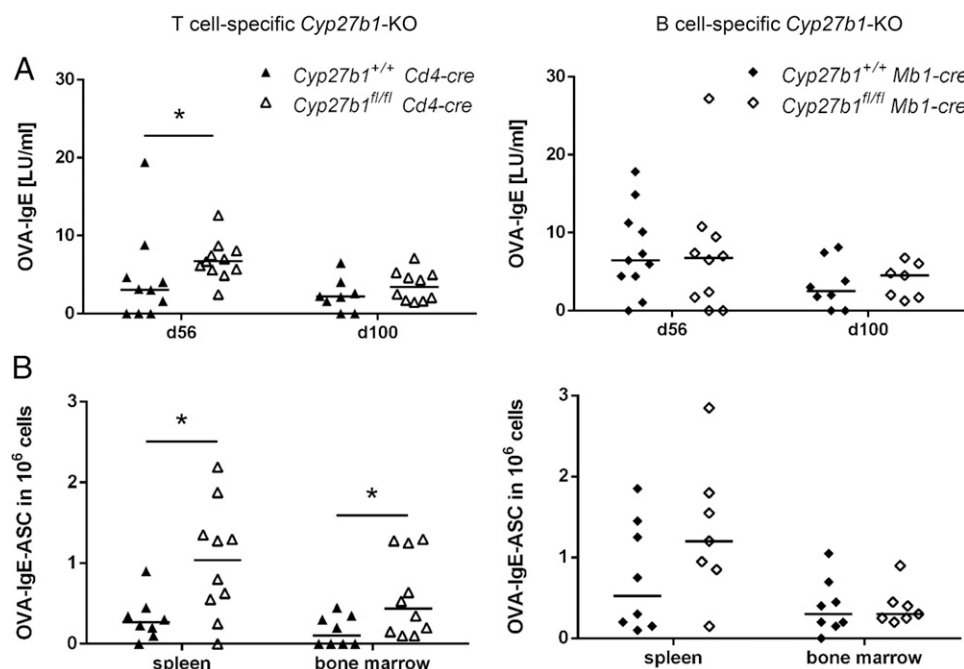


FIGURE 4. Increased humoral IgE response in T cell-specific *Cyp27b1*-KO mice. T cell- or B cell-specific *Cyp27b1*-KO mice were generated by crossing mice expressing Cre-recombinase under transcriptional control of *Cd4* or *Mb1* promoter with *Cyp27b1* exon 8-floxed mice. The mice were sensitized and challenged as described in Fig. 1. Plasma Ig concentrations (A) and (B) numbers of ASC were determined by ELISA and ELISpot regarding OVA-IgE. The lines indicate the median of 7–11 mice. * $p \leq 0.05$.

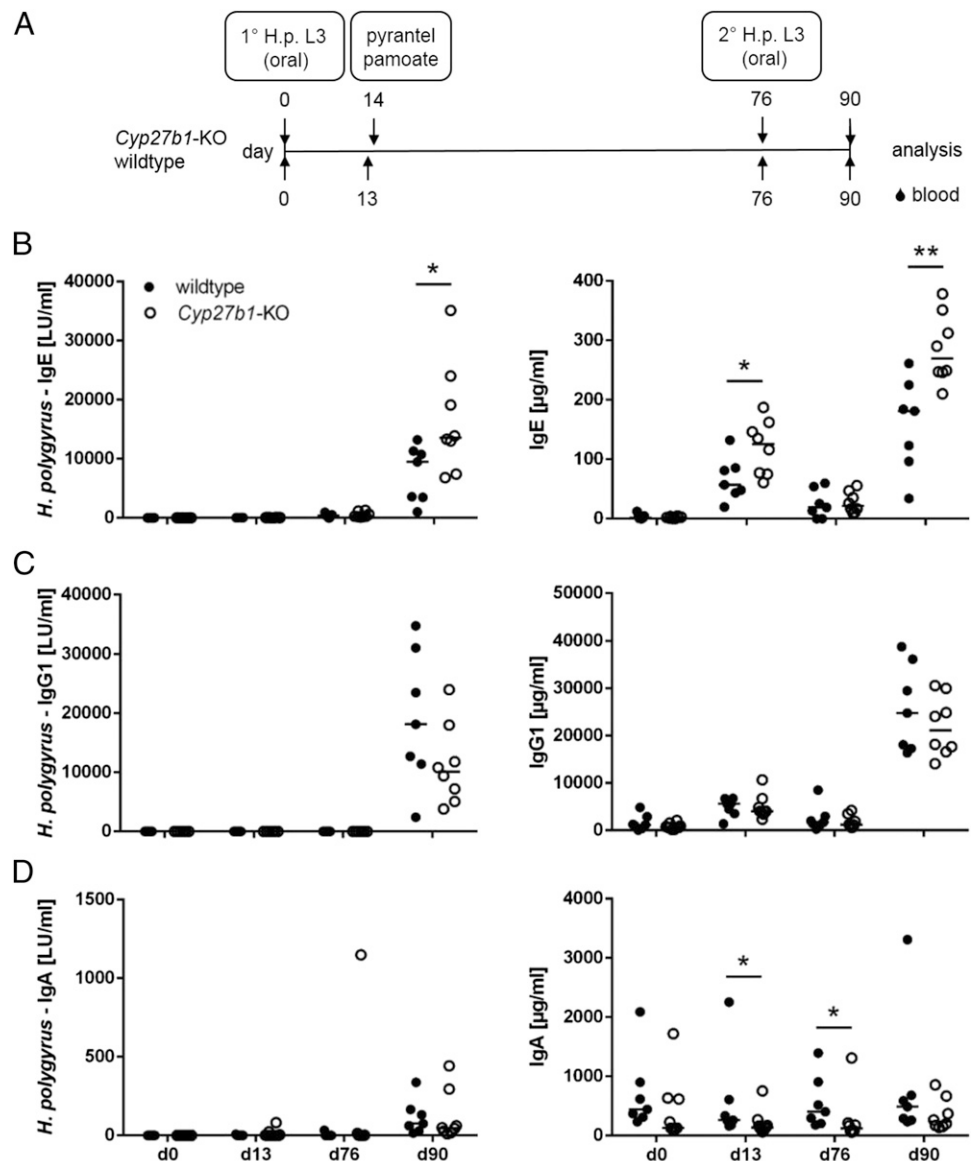


FIGURE 5. Increased IgE response in *Cyp27b1*-KO mice following intestinal helminth infection. **(A)** Experimental setup. Primary infection of *Cyp27b1*-KO and wildtype mice with *H. polygyrus* was abrogated on day 14 by pyrantel treatment. After reinfection on day 76, the immunological memory response was determined on day 90. The humoral immune response was analyzed over time regarding plasma Ig concentrations of *H. polygyrus*-specific and total **(B)** IgE, **(C)** IgG1, and **(D)** IgA by ELISA. The lines indicate the median of $n = 7$ –8 mice. * $p \leq 0.05$, ** $p \leq 0.01$.

induction model, i.e., gastrointestinal infection with the parasite *H. polygyrus*, we confirm that endogenous calcitriol participates in the control of specific IgE responses. The data of the cell-specific KO mice show a role of *Cyp27b1* directly in T lymphocytes to negatively regulate the IgE response, and by trend also in B cells.

As IgE regulation was independent of the inducing signals, namely OVA sensitization or parasite infection, a common signaling cascade is likely, e.g., control of IgE class-switch recombination. Previously it was shown that VDR activation inhibits IgE switch transcript expression (4) by recruitment of a *trans*-repressive VDR-DNA complex (26). In addition, other important molecules for IgE class-switching are targeted by VDR as shown before by reduced NF- κ B activation and expression of its target in B cells, *aicda* (4, 6, 27). These data were confirmed in mice using a synthetic low-calcemic VDR agonist (6) or VDR-KO mice (7).

Also, specific IgG1 and IgA were increased in the plasma of sensitized *Cyp27b1*-KO mice. The data suggest that endogenous calcitriol controls these Ig classes, beyond specific mechanisms as shown for IgE, by reducing the T cell reactivity or general mechanisms of the class-switch machinery in mice such as downregulation of AID as shown by us previously (6). In addition, IgE induction by sensitization and helminth infection follows

different signals, e.g., induction of Th2 cells by thymic stromal lymphopoietin or thymic stromal lymphopoietin-independent suppression of Th1 polarization, respectively (28, 29). Thus, our data support that endogenous calcitriol synthesis reduces T cell costimulation and/or central molecules in the isotype switching process, such as AID and NF- κ B in B cells.

We identified that specific IgE concentrations are also increased in mice with T cell-specific *Cyp27b1*-KO as well as the numbers of OVA-IgE secreting cells. This supports the functional relevance of the *Cyp27b1* mRNA expression in lymphocytes. Our data support that although kidney-derived calcitriol will contribute to most of the serum calcitriol (physiologic range of 25–45 pg/ml) (30), these concentrations are below the VDR-activating threshold in B cells (4, 6). Thus, endogenous calcitriol from activated immune cells in lymphoid tissues is important in a paracrine and/or autocrine fashion for the immune functions of calcitriol, as discussed previously (1, 31, 32) and extending the knowledge to IgE regulation by genetic evidence. Moreover, VDR function in murine B lymphocytes, but not T lymphocytes, contributes to the control of sensitization-induced specific IgE, as shown recently in lymphocyte-specific KO mice (33). Thus, our data suggest that endogenous calcitriol from T cells acts on the VDR in B cells to

reduce IgE in vivo. However, endogenous calcitriol derived from other immune cells and/or the kidney contributes to IgE regulation, as the calcitriol effect was not abolished completely by lymphocyte-specific knockout.

In addition to a function of endogenous calcitriol in the isotype switching, we determined altered frequencies of OVA-specific ASC in the spleen and bone marrow. Our data support the previous finding that endogenous calcitriol can alter the migration of specific immune cells, however, the general trafficking into spleen is not affected (34). In contrast to a previous report on reduced differentiation of B cells to Ab-secreting human plasmablasts (35), we detected normal frequencies in mature B cell subsets and plasma cells in aged *Cyp27b1*-KO mice. Only B cell progenitors in the bone marrow were marginally reduced, potentially through preclinical bone abnormalities such as osteomalacia (36). Supporting our data, the immune cell compartments are grossly intact in *Cyp27b1*-KO mice of a different genetic background (37). In addition, we showed increased numbers of OVA-IgG1 ASCs in the spleen of *Cyp27b1*-KO mice along with strong enhancement of Ig production, supporting and confirming previous data that VDR is not influencing the plasma cell differentiation as such, but rather specific activation and differentiation processes including IgE class-switch. However, B cell-independent mechanisms of IgE control by endogenous calcitriol cannot be excluded by our data, e.g., through regulatory T cell differentiation by calcitriol-primed dendritic cells (38, 39) and/or IL-10-secreting Tr1 cells (40). Although Th2 polarization by VDR including from VDR-KO mice was suggested by previous reports (7), our data do not support a strong Th2 differentiation, as the IgE-response was increased in the absence of endogenous calcitriol.

In conclusion, in this study we show that endogenous calcitriol reduces the humoral IgE and IgG1 response to OVA sensitization or parasite infection. Therefore, vitamin D deficiency should be avoided to take advantage of the beneficial effects of endogenous calcitriol regarding bone health and IgE regulation.

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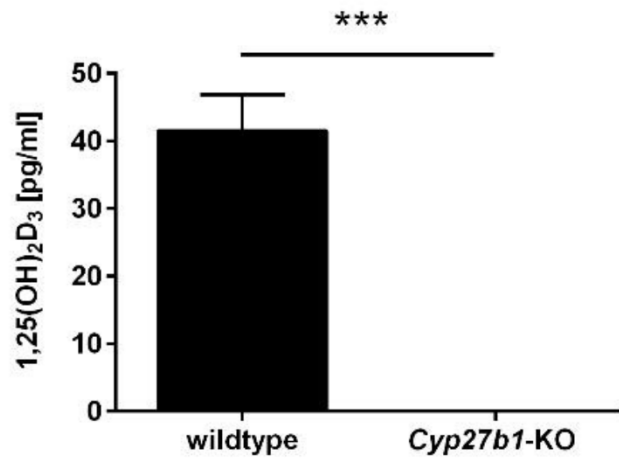
Disclosures

The authors have no financial conflicts of interest.

References

- Adams, J. S., and M. Hewison. 2008. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat. Clin. Pract. Endocrinol. Metab.* 4: 80–90.
- Provvedini, D. M., C. D. Tsoukas, L. J. Deftos, and S. C. Manolagas. 1983. 1,25-dihydroxyvitamin D₃ receptors in human leukocytes. *Science* 221: 1181–1183.
- Morgan, J. W., D. M. Morgan, S. R. Lasky, D. Ford, N. Kouttab, and A. L. Maizel. 1996. Requirements for induction of vitamin D-mediated gene regulation in normal human B lymphocytes. *J. Immunol.* 157: 2900–2908.
- Heine, G., K. Anton, B. M. Henz, and M. Worm. 2002. 1 α ,25-dihydroxyvitamin D₃ inhibits anti-CD40 plus IL-4-mediated IgE production in vitro. *Eur. J. Immunol.* 32: 3395–3404.
- Milovanovic, M., G. Heine, W. Hallatschek, B. Opitz, A. Radbruch, and M. Worm. 2010. Vitamin D receptor binds to the epsilon germline gene promoter and exhibits transrepressive activity. *J. Allergy Clin. Immunol.* 126: 1016–1023, 1023 e1011–1014.
- Hartmann, B., G. Heine, M. Babina, A. Steinmeyer, U. Zügel, A. Radbruch, and M. Worm. 2011. Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response. *Allergy* 66: 540–548.
- Wittke, A., V. Weaver, B. D. Mahon, A. August, and M. T. Cantorna. 2004. Vitamin D receptor-deficient mice fail to develop experimental allergic asthma. *J. Immunol.* 173: 3432–3436.
- Sigmundsdottir, H., J. Pan, G. F. Debes, C. Alt, A. Habtezion, D. Soler, and E. C. Butcher. 2007. DCs metabolize sunlight-induced vitamin D₃ to 'program' T cell attraction to the epidermal chemokine CCL27. *Nat. Immunol.* 8: 285–293.
- Drozdenko, G., G. Heine, and M. Worm. 2014. Oral vitamin D increases the frequencies of CD38+ human B cells and ameliorates IL-17-producing T cells. *Exp. Dermatol.* 23: 107–112.
- Heine, G., U. Niesner, H. D. Chang, A. Steinmeyer, U. Zügel, T. Zuberbier, A. Radbruch, and M. Worm. 2008. 1,25-dihydroxyvitamin D₃ promotes IL-10 production in human B cells. *Eur. J. Immunol.* 38: 2210–2218.
- Hyppönen, E., D. J. Berry, M. Wjst, and C. Power. 2009. Serum 25-hydroxyvitamin D and IgE - a significant but nonlinear relationship. *Allergy* 64: 613–620.
- Brehm, J. M., J. C. Celedón, M. E. Soto-Quiros, L. Avila, G. M. Hunninghake, E. Forno, D. Laskey, J. S. Sylvia, B. W. Hollis, S. T. Weiss, and A. A. Litonjua. 2009. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am. J. Respir. Crit. Care Med.* 179: 765–771.
- Heine, G., C. Tabeling, B. Hartmann, C. R. González Calera, A. A. Kühl, J. Lindner, A. Radbruch, M. Witznath, and M. Worm. 2014. 25-hydroxyvitamin D₃ promotes the long-term effect of specific immunotherapy in a murine allergy model. *J. Immunol.* 193: 1017–1023.
- Dardenne, O., J. Prud'homme, A. Arabian, F. H. Glorieux, and R. St-Arnaud. 2001. Targeted inactivation of the 25-hydroxyvitamin D₃-1 α (α)-hydroxylase gene (CYP27B1) creates an animal model of pseudovitamin D-deficiency rickets. *Endocrinology* 142: 3135–3141.
- Bikle, D. D., S. Chang, D. Crumrine, H. Elalieh, M. Q. Man, O. Dardenne, Z. Xie, R. S. Arnaud, K. Feingold, and P. M. Elias. 2004. Mice lacking 25OHD 1 α -hydroxylase demonstrate decreased epidermal differentiation and barrier function. *J. Steroid Biochem. Mol. Biol.* 89–90: 347–353.
- Hobeika, E., S. Thiemann, B. Storch, H. Jumaa, P. J. Nielsen, R. Pelanda, and M. Reth. 2006. Testing gene function early in the B cell lineage in mb1-cre mice. *Proc. Natl. Acad. Sci. USA* 103: 13789–13794.
- Grivennikov, S. I., A. V. Tumanov, D. J. Liepinsh, A. A. Kruglov, B. I. Marakusha, A. N. Shakhov, T. Murakami, L. N. Drutskaia, I. Förster, B. E. Clausen, et al. 2005. Distinct and nonredundant in vivo functions of TNF produced by T cells and macrophages/neutrophils: protective and deleterious effects. *Immunity* 22: 93–104.
- Simmons, S., M. Knoll, C. Drewell, I. Wolf, H. J. Mollenkopf, C. Bouquet, and F. Melchers. 2012. Biphentotypic B-lymphoid/myeloid cells expressing low levels of Pax5: potential targets of BAL development. *Blood* 120: 3688–3698.
- Heine, G., A. Dahten, K. Hilt, D. Ernst, M. Milovanovic, B. Hartmann, and M. Worm. 2009. Liver X receptors control IgE expression in B cells. *J. Immunol.* 182: 5276–5282.
- Dahten, A., C. Koch, D. Ernst, C. Schnöller, S. Hartmann, and M. Worm. 2008. Systemic PPAR γ ligand inhibits allergic immune response in the skin. *J. Invest. Dermatol.* 128: 2211–2218.
- Rzepecka, J., R. Lucius, M. Doligalska, S. Beck, S. Rausch, and S. Hartmann. 2006. Screening for immunomodulatory proteins of the intestinal parasitic nematode *Heligmosomoides polygyrus*. *Parasite Immunol.* 28: 463–472.
- Mudnakudu Nagaraju, K. K., M. Babina, and M. Worm. 2013. Opposing effects on immune function and skin barrier regulation by the proteasome inhibitor bortezomib in an allergen-induced eczema model. *Exp. Dermatol.* 22: 742–747.
- Radbruch, A., G. Muehlinghaus, E. O. Luger, A. Inamine, K. G. Smith, T. Dörner, and F. Hiepe. 2006. Competence and competition: the challenge of becoming a long-lived plasma cell. *Nat. Rev. Immunol.* 6: 741–750.
- Luger, E. O., V. Fokuhl, M. Wegmann, M. Abram, K. Tillack, G. Achatz, R. A. Manz, M. Worm, A. Radbruch, and H. Renz. 2009. Induction of long-lived allergen-specific plasma cells by mucosal allergen challenge. *J. Allergy Clin. Immunol.* 124: 819–826.e814.
- Geha, R. S., H. H. Jabara, and S. R. Brodeur. 2003. The regulation of immunoglobulin E class-switch recombination. *Nat. Rev. Immunol.* 3: 721–732.
- Milovanovic, M., G. Drozdenko, C. Weise, M. Babina, and M. Worm. 2010. Interleukin-17A promotes IgE production in human B cells. *J. Invest. Dermatol.* 130: 2621–2628.
- Geldmeyer-Hilt, K., G. Heine, B. Hartmann, R. Baumgrass, A. Radbruch, and M. Worm. 2011. 1,25-dihydroxyvitamin D₃ impairs NF- κ B activation in human naïve B cells. *Biochem. Biophys. Res. Commun.* 407: 669–702.
- Massacand, J. C., R. C. Stettler, R. Meier, N. E. Humphreys, R. K. Grencis, B. J. Marsland, and N. L. Harris. 2009. Helminth products bypass the need for TSLP in Th2 immune responses by directly modulating dendritic cell function. *Proc. Natl. Acad. Sci. USA* 106: 13968–13973.
- Leyva-Castillo, J. M., P. Hener, H. Jiang, and M. Li. 2013. TSLP produced by keratinocytes promotes allergen sensitization through skin and thereby triggers atopic march in mice. *J. Invest. Dermatol.* 133: 154–163.
- Kratz, A., M. Ferraro, P. M. Sluss, and K. B. Lewandrowski. 2004. Case records of the Massachusetts general hospital. Weekly clinicopathological exercises. Laboratory reference values. *N. Engl. J. Med.* 351: 1548–1563.
- Hewison, M. 2012. An update on vitamin D and human immunity. *Clin. Endocrinol. (Oxf.)* 76: 315–325.
- Mora, J. R., M. Iwata, and U. H. von Andrian. 2008. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat. Rev. Immunol.* 8: 685–698.
- James, J., V. Weaver, and M. T. Cantorna. 2017. Control of circulating IgE by the vitamin D receptor in vivo involves B cell intrinsic and extrinsic mechanisms. *J. Immunol.* 198: 1164–1171.
- Enioutina, E. Y., D. Bareyan, and R. A. Daynes. 2009. TLR-induced local metabolism of vitamin D₃ plays an important role in the diversification of adaptive immune responses. *J. Immunol.* 182: 4296–4305.

35. Chen, S., G. P. Sims, X. X. Chen, Y. Y. Gu, S. Chen, and P. E. Lipsky. 2007. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J. Immunol.* 179: 1634–1647.
36. Dardenne, O., J. Prud'homme, S. A. Hacking, F. H. Glorieux, and R. St-Arnaud. 2003. Correction of the abnormal mineral ion homeostasis with a high-calcium, high-phosphorus, high-lactose diet rescues the PDDR phenotype of mice deficient for the 25-hydroxyvitamin D-1alpha-hydroxylase (CYP27B1). *Bone* 32: 332–340.
37. Panda, D. K., D. Miao, M. L. Tremblay, J. Sirois, R. Farookhi, G. N. Hendy, and D. Goltzman. 2001. Targeted ablation of the 25-hydroxyvitamin D 1alpha -hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *Proc. Natl. Acad. Sci. USA* 98: 7498–7503.
38. Adorini, L. 2003. Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting autoimmune diabetes. *Ann. N. Y. Acad. Sci.* 987: 258–261.
39. Penna, G., and L. Adorini. 2000. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J. Immunol.* 164: 2405–2411.
40. Barrat, F. J., D. J. Cua, A. Boonstra, D. F. Richards, C. Crain, H. F. Savelkoul, R. de Waal-Malefyt, R. L. Coffman, C. M. Hawrylowicz, and A. O'Garra. 2002. In vitro generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J. Exp. Med.* 195: 603–616.



Supplementary Figure 1. Calcitriol deficiency in *Cyp27b1*-KO mice. Plasma calcitriol concentrations in *Cyp27b1*-KO and wildtype mice. Data are shown as mean ± SEM, n=6. *** $p \leq 0.001$.

Cell type	Organ	Median cell count		Median frequency		Significance
		<i>Cyp27b1</i> -KO	wildtype	<i>Cyp27b1</i> -KO	wildtype	
CD19 ⁺ AA4.1 ⁺ cKit ⁺ pro B cells (1, 2)	Bone marrow	2.93 x 10 ³	1.06 x 10 ⁴	0.8	3.0	NS
	Peritoneum	1.14 x 10 ²	1.39 x 10 ²	0.2	0.0	NS
	Spleen	9.78 x 10 ²	5.96 x 10 ²	0.1	0.0	NS
CD19 ⁺ AA4.1 ⁺ cKit ⁺ pre B cells (1, 2)	Bone marrow	3.90 x 10 ⁴	6.50 x 10 ⁴	9.4	24.7	NS
	Peritoneum	3.23 x 10 ²	4.80 x 10 ²	0.4	0.2	NS
	Spleen	1.24 x 10 ⁴	2.40 x 10 ⁴	1.0	1.1	NS
CD19 ⁺ AA4.1 ⁺ CD25 ⁻ pro B / pre B-1 (3)	Bone marrow	2.90 x 10 ⁴	6.04 x 10 ⁴	7.0	17.6	NS
	Peritoneum	4.06 x 10 ²	5.68 x 10 ²	0.6	0.2	NS
	Spleen	8.80 x 10 ³	2.06 x 10 ⁴	1.2	1.0	NS
CD19 ⁺ AA4.1 ⁺ CD25 ⁺ pre B / pre B-2 (3)	Bone marrow	1.14 x 10 ⁴	1.75 x 10 ⁴	3.0	7.8	NS
	Peritoneum	3.72 x 10 ¹	4.00 x 10 ¹	0.0	0.0	NS
	Spleen	5.15 x 10 ²	7.15 x 10 ²	0.1	0.0	NS
CD19 ⁺ CD5 ⁺ IgM ^{high} B-1a (4)	Bone marrow	2.38 x 10 ⁴	6.92 x 10 ³	7.1	1.8	NS
	Peritoneum	1.28 x 10 ⁴	3.71 x 10 ⁴	14.1	8.2	NS
	Spleen	4.50 x 10 ⁴	5.44 x 10 ⁴	5.3	2.9	NS
CD19 ⁺ IgM ⁺ IgD ^{low} B-1b (4)	Bone marrow	1.24 x 10 ⁴	1.68 x 10 ⁴	3.7	6.2	NS
	Peritoneum	2.78 x 10 ⁴	6.60 x 10 ⁴	24.2	14.6	NS
	Spleen	4.98 x 10 ⁴	1.52 x 10 ⁵	7.1	7.5	NS
CD19 ⁺ CD5 ⁻ IgD ^{high} follicular B-2 (4)	Bone marrow	2.63 x 10 ⁵	1.62 x 10 ⁵	78.9	63.6	NS
	Peritoneum	3.77 x 10 ⁴	3.23 x 10 ⁵	57.5	71.2	NS
	Spleen	7.83 x 10 ⁵	1.56 x 10 ⁶	84.9	76.4	NS
CD19 ⁺ IgD ⁺ IgM ^{low} mature B-2 (2)	Bone marrow	2.43 x 10 ⁵	1.39 x 10 ⁵	73.0	52.9	NS
	Peritoneum	3.49 x 10 ⁴	2.88 x 10 ⁵	54.2	62.9	NS
	Spleen	6.86 x 10 ⁵	1.31 x 10 ⁶	74.5	71.2	NS
CD19 ⁺ B cells (5, 6)	Bone marrow	3.31 x 10 ⁵	2.64 x 10 ⁵	19.9	19.5	NS
	Peritoneum	7.87 x 10 ⁴	4.22 x 10 ⁵	57.3	61.9	NS
	Thymus	8.74 x 10 ³	5.24 x 10 ⁴	0.4	0.5	NS
	Spleen	9.30 x 10 ⁵	2.04 x 10 ⁶	30.1	47.9	NS

Cell type	Organ	Median cell count		Median frequency		Significance
		<i>Cyp27b1</i> -KO	wildtype	<i>Cyp27b1</i> -KO	wildtype	
CD19 ⁺ IgM ⁺ IgD ⁻ immature B cells (7)	Bone marrow	1.53 x 10 ⁴	1.09 x 10 ⁵	5.0	42.6	NS
	Peritoneum	3.59 x 10 ⁴	9.05 x 10 ⁴	32.3	21.5	NS
	Thymus	6.90 x 10 ²	3.76 x 10 ³	6.6	7.2	NS
	Spleen	4.76 x 10 ⁴	1.91 x 10 ⁵	6.8	9.4	NS
CD19 ⁺ CD21 ⁻ CD23 ⁻ transitional type 1 (8)	Bone marrow	7.98 x 10 ⁴	1.31 x 10 ⁵	24.1	44.5	NS
	Peritoneum	5.36 x 10 ⁴	1.74 x 10 ⁵	53.4	41.4	NS
	Thymus	5.01 x 10 ³	2.09 x 10 ⁴	46.5	55.0	NS
	Spleen	1.23 x 10 ⁵	2.30 x 10 ⁵	13.2	12.9	NS
CD19 ⁺ CD21 ⁺ CD23 ⁺ transitional type 2 (8)	Bone marrow	5.58 x 10 ³	7.98 x 10 ³	1.5	2.3	NS
	Peritoneum	5.28 x 10 ²	6.62 x 10 ²	0.5	0.2	NS
	Thymus	1.76 x 10 ¹	1.28 x 10 ²	0.4	0.3	NS
	Spleen	6.21 x 10 ⁴	1.33 x 10 ⁵	6.3	6.1	NS
CD21 ⁺ CD23 ⁻ marginal zone B cells (8)	Bone marrow	7.23 x 10 ²	2.13 x 10 ³	0.2	0.6	NS
	Peritoneum	1.12 x 10 ²	1.10 x 10 ²	0.1	0.0	NS
	Thymus	0	0	0	0	NS
	Spleen	9.24 x 10 ⁴	2.81 x 10 ⁵	13.2	11.6	NS
CD19 ⁺ IgM ⁺ IgD ⁺ mature B cells (7)	Bone marrow	3.40 x 10 ⁴	1.46 x 10 ⁴	10.0	4.8	NS
	Peritoneum	7.33 x 10 ³	4.48 x 10 ⁴	7.0	10.6	NS
	Thymus	7.78 x 10 ²	7.16 x 10 ²	6.7	1.2	NS
	Spleen	6.72 x 10 ⁴	1.20 x 10 ⁵	6.9	6.3	NS
CD19 ⁺ IgM ⁻ IgD ⁻ switched B cells(9)	Bone marrow	2.05 x 10 ³	6.95 x 10 ³	23.3	46.6	NS
	Peritoneum	3.78 x 10 ²	4.31 x 10 ²	26.1	23.7	NS
	Thymus	9.28 x 10 ²	7.82 x 10 ³	48.7	53.8	NS
	Spleen	1.27 x 10 ³	8.39 x 10 ³	16.5	23.3	NS

Suppl. Tab. I: Flow-cytometric analysis of B cell subsets at different stages of development (immature, B-1 and B-2, mature) in tissues of *Cyp27b1*-KO mice and wildtype littermates (n=3). Median frequencies of *Cyp27b1*-KO and wildtype mice were analyzed by Mann-Whitney *U* test. NS, not significant.

Cell type	Organ	Median cell count		Median frequency		Significance
		<i>Cyp27b1</i> -KO	wildtype	<i>Cyp27b1</i> -KO	wildtype	
CD4 ⁺ T cells (10)	Bone marrow	1.50 x 10 ⁵	1.02 x 10 ⁵	12.3	8.4	NS
	Thymus	8.40 x 10 ⁴	5.38 x 10 ⁵	4.9	9.1	NS
	Spleen	4.56 x 10 ⁵	1.20 x 10 ⁶	21.0	24.2	NS
CD4 ⁺ CD25 ⁺ regulatory T cells (11)	Bone marrow	2.35 x 10 ⁴	1.78 x 10 ⁴	1.8	1.7	NS
	Thymus	2.19 x 10 ⁴	8.47 x 10 ⁴	1.8	0.8	NS
	Spleen	6.29 x 10 ⁴	1.24 x 10 ⁵	2.1	2.7	NS
CD4 ⁺ CD44 ⁺ memory T cells (12)	Bone marrow	3.00 x 10 ⁴	2.95 x 10 ⁴	2.3	2.7	NS
	Thymus	6.83 x 10 ³	7.55 x 10 ⁴	0.4	0.6	NS
	Spleen	7.58 x 10 ⁴	1.25 x 10 ⁵	3.5	2.2	NS
CD8 ⁺ cytotoxic T cells (13)	Bone marrow	1.55 x 10 ⁵	7.20 x 10 ⁴	12.7	6.1	NS
	Thymus	1.29 x 10 ⁵	6.48 x 10 ⁵	4.0	3.8	NS
	Spleen	7.19 x 10 ⁵	9.35 x 10 ⁵	29.2	17.7	NS
CD8 ⁺ CD25 ⁺ regulatory T cells (14)	Bone marrow	5.72 x 10 ²	3.20 x 10 ²	0.0	0.0	NS
	Thymus	2.45 x 10 ⁴	7.27 x 10 ⁴	2.1	0.6	NS
	Spleen	4.24 x 10 ³	4.32 x 10 ³	0.1	0.1	NS
CD8 ⁺ CD44 ⁺ memory T cells (12)	Bone marrow	1.76 x 10 ⁴	1.69 x 10 ⁴	1.4	1.4	NS
	Thymus	3.05 x 10 ³	3.89 x 10 ⁴	0.2	0.2	NS
	Spleen	3.16 x 10 ⁴	9.31 x 10 ⁴	1.3	1.6	NS
CD4 ⁺ CD8 ⁺ double positive T cells (15)	Bone marrow	1.77 x 10 ³	7.26 x 10 ³	0.1	0.7	NS
	Thymus	9.29 x 10 ⁵	9.33 x 10 ⁶	79.8	86.5	NS
	Spleen	4.30 x 10 ³	1.44 x 10 ⁴	0.2	0.3	NS

Suppl. Tab. II: Flow-cytometric analysis of T cell subsets in tissues of *Cyp27b1*-KO mice and wildtype littermates (n=3). Median frequencies of *Cyp27b1*-KO and wildtype mice were analyzed by Mann-Whitney *U* test. NS, not significant.

Cell type	Organ	Median cell count		Median frequency		Significance
		<i>Cyp27b1</i> -KO	wildtype	<i>Cyp27b1</i> -KO	wildtype	
CD19 ⁻ CD11b ^{high} macrophages (16)	Bone marrow	2.07 x 10 ⁵	3.06 x 10 ⁵	8.1	10.0	NS
	Peritoneum	1.33 x 10 ⁵	2.98 x 10 ⁵	64.7	50.4	NS
	Thymus	1.90 x 10 ³	3.46 x 10 ⁴	0.2	0.2	NS
	Spleen	5.80 x 10 ⁴	6.00 x 10 ⁴	1.7	1.9	NS
CD19 ⁻ Gr-1 ⁺ granulocytes (17)	Bone marrow	7.51 x 10 ⁵	1.33 x 10 ⁶	31.6	42.7	NS
	Spleen	5.28 x 10 ⁴	8.28 x 10 ⁴	2.5	3.6	NS
CD19 ⁻ CD11b ⁺ Gr-1 ⁺ neutrophils (18) / myeloid suppressor cells (19)	Bone marrow	6.19 x 10 ⁵	1.20 x 10 ⁶	19.1	16.1	NS
	Spleen	3.29 x 10 ⁴	6.37 x 10 ⁴	33.3	14.3	NS
CD19 ⁻ CD11b ^{hi} Gr-1 ⁺ (20)	Bone marrow	1.27 x 10 ⁵	1.75 x 10 ⁵	72.4	78.0	NS
	Spleen	8.39 x 10 ³	1.19 x 10 ⁴	45.2	74.8	NS
CD19 ⁻ CD11b ^{int} Gr-1 ^{low} (20)	Bone marrow	1.58 x 10 ⁵	2.73 x 10 ⁵	6.5	7.4	NS
	Spleen	4.20 x 10 ³	7.30 x 10 ⁴	0.3	2.3	NS
CD19 ⁻ CD11b ^{int} Gr-1 ^{hi} (20)	Bone marrow	7.37 x 10 ⁵	1.31 x 10 ⁶	29.6	39.7	NS
	Spleen	2.60 x 10 ⁴	1.02 x 10 ⁵	1.6	3.9	NS
CD19 ⁻ CD11c ⁺ Gr-1 ^{neg} dendritic cells (21)	Bone marrow	7.29 x 10 ⁴	1.78 x 10 ⁵	2.7	4.4	NS
	Peritoneum	4.56 x 10 ³	1.10 x 10 ⁴	2.7	2.0	NS
	Thymus	1.52 x 10 ⁴	9.68 x 10 ⁴	0.9	0.4	NS
	Spleen	1.43 x 10 ⁵	1.12 x 10 ⁵	8.1	4.2	NS
CD19 ⁻ CD11c ⁺ Gr-1 ⁺ plasmacytoid dendritic cells (22)	Bone marrow	2.56 x 10 ⁴	3.78 x 10 ⁴	1.0	1.0	NS
	Spleen	1.43 x 10 ⁴	2.09 x 10 ⁴	0.4	0.8	NS

Suppl. Tab. III: Flow-cytometric analysis of myeloid cell subsets in tissues of *Cyp27b1*-KO mice and wildtype littermates (n=3). Median frequencies of *Cyp27b1*-KO and wildtype mice were analyzed by Mann-Whitney *U* test. NS, not significant.

References Supplemental Data

1. Hardy, R. R., and K. Hayakawa. 2001. B cell development pathways. *Annual review of immunology* 19: 595-621.
2. Nagasawa, T. 2006. Microenvironmental niches in the bone marrow required for B-cell development. *Nature reviews. Immunology* 6: 107-116.
3. Hardy, R. R., P. W. Kincade, and K. Dorshkind. 2007. The protean nature of cells in the B lymphocyte lineage. *Immunity* 26: 703-714.
4. Baumgarth, N. 2011. The double life of a B-1 cell: self-reactivity selects for protective effector functions. *Nature reviews. Immunology* 11: 34-46.
5. Tedder, T. F. 2009. CD19: a promising B cell target for rheumatoid arthritis. *Nature reviews. Rheumatology* 5: 572-577.
6. Otero, D. C., and R. C. Rickert. 2003. CD19 function in early and late B cell development. II. CD19 facilitates the pro-B/pre-B transition. *J Immunol* 171: 5921-5930.
7. Yabas, M., C. E. Teh, S. Frankenreiter, D. Lal, C. M. Roots, B. Whittle, D. T. Andrews, Y. Zhang, N. C. Teoh, J. Sprent, L. E. Tze, E. M. Kucharska, J. Kofler, G. C. Farrell, S. Broer, C. C. Goodnow, and A. Enders. 2011. ATP11C is critical for the internalization of phosphatidylserine and differentiation of B lymphocytes. *Nature immunology* 12: 441-449.
8. Shen, P., and S. Fillatreau. 2015. Antibody-independent functions of B cells: a focus on cytokines. *Nature reviews. Immunology* 15: 441-451.
9. McHeyzer-Williams, L. J., P. J. Milpied, S. L. Okitsu, and M. G. McHeyzer-Williams. 2015. Class-switched memory B cells remodel BCRs within secondary germinal centers. *Nature immunology* 16: 296-305.
10. Zhu, J., and W. E. Paul. 2008. CD4 T cells: fates, functions, and faults. *Blood* 112: 1557-1569.
11. Xu, D., H. Liu, M. Komai-Koma, C. Campbell, C. McSharry, J. Alexander, and F. Y. Liew. 2003. CD4+CD25+ regulatory T cells suppress differentiation and functions of Th1 and Th2 cells, Leishmania major infection, and colitis in mice. *J Immunol* 170: 394-399.
12. Dutt, S., J. Baker, H. E. Kohrt, N. Kambham, M. Sanyal, R. S. Negrin, and S. Strober. 2011. CD8+CD44(hi) but not CD4+CD44(hi) memory T cells mediate potent graft antilymphoma activity without GVHD. *Blood* 117: 3230-3239.
13. Yuzefpolskiy, Y., F. M. Baumann, V. Kalia, and S. Sarkar. 2015. Early CD8 T-cell memory precursors and terminal effectors exhibit equipotent in vivo degranulation. *Cellular & molecular immunology* 12: 400-408.
14. Xie, Y., X. Zhang, T. Zhao, W. Li, and J. Xiang. 2013. Natural CD8(+)25(+) regulatory T cell-secreted exosomes capable of suppressing cytotoxic T lymphocyte-mediated immunity against B16 melanoma. *Biochemical and biophysical research communications* 438: 152-155.
15. Zuckermann, F. A. 1999. Extrathymic CD4/CD8 double positive T cells. *Veterinary immunology and immunopathology* 72: 55-66.
16. Bain, C. C., A. Bravo-Blas, C. L. Scott, E. Gomez Perdiguero, F. Geissmann, S. Henri, B. Malissen, L. C. Osborne, D. Artis, and A. M. Mowat. 2014. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nature immunology* 15: 929-937.
17. Jiang, D., and H. Schwarz. 2010. Regulation of granulocyte and macrophage populations of murine bone marrow cells by G-CSF and CD137 protein. *PloS one* 5: e15565.

18. Jonsson, F., D. A. Mancardi, Y. Kita, H. Karasuyama, B. Iannascoli, N. Van Rooijen, T. Shimizu, M. Daeron, and P. Bruhns. 2011. Mouse and human neutrophils induce anaphylaxis. *The Journal of clinical investigation* 121: 1484-1496.
19. Makarenkova, V. P., V. Bansal, B. M. Matta, L. A. Perez, and J. B. Ochoa. 2006. CD11b+/Gr-1+ myeloid suppressor cells cause T cell dysfunction after traumatic stress. *J Immunol* 176: 2085-2094.
20. Zhu, B., Y. Bando, S. Xiao, K. Yang, A. C. Anderson, V. K. Kuchroo, and S. J. Khoury. 2007. CD11b+Ly-6C(hi) suppressive monocytes in experimental autoimmune encephalomyelitis. *J Immunol* 179: 5228-5237.
21. Shortman, K., and Y. J. Liu. 2002. Mouse and human dendritic cell subtypes. *Nature reviews. Immunology* 2: 151-161.
22. Ferrero, I., W. Held, A. Wilson, F. Tacchini-Cottier, F. Radtke, and H. R. MacDonald. 2002. Mouse CD11c(+) B220(+) Gr1(+) plasmacytoid dendritic cells develop independently of the T-cell lineage. *Blood* 100: 2852-2857.