

Accepted Manuscript

B cells control maternofetal priming of allergy and tolerance in a murine model of allergic airway inflammation

Christine Happle, MD/PhD, Adan Chari Jirmo, PhD, Almut Meyer-Bahlburg, MD, Anika Habener, PhD, Heinz Gerd Hoymann, PhD, Christian Hennig, MD, Jelena Skuljec, PhD, Gesine Hansen, MD

PII: S0091-6749(17)30920-X

DOI: [10.1016/j.jaci.2017.03.051](https://doi.org/10.1016/j.jaci.2017.03.051)

Reference: YMAI 12847

To appear in: *Journal of Allergy and Clinical Immunology*

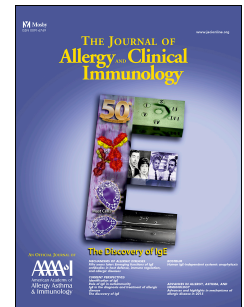
Received Date: 13 July 2016

Revised Date: 25 February 2017

Accepted Date: 27 March 2017

Please cite this article as: Happle C, Jirmo AC, Meyer-Bahlburg A, Habener A, Hoymann HG, Hennig C, Skuljec J, Hansen G, B cells control maternofetal priming of allergy and tolerance in a murine model of allergic airway inflammation, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.03.051.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



B cells control maternofetal priming of allergy and tolerance in a murine model of allergic airway inflammation

Christine Happle MD/PhD^{1,2}, Adan Chari Jirmo PhD^{1,2}, Almut Meyer-Bahlburg MD^{1,2,3}, Anika Habener PhD^{1,2}, Heinz Gerd Hoymann PhD⁴, Christian Hennig MD^{1,2}, Jelena Skuljec PhD¹, Gesine Hansen MD^{1,2}

¹Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany

²Biomedical Research in Endstage and Obstructive Lung Disease (BREATH), Member of the German Center for Lung Research (DZL)

³Department of Pediatrics, University Medicine Greifswald, Greifswald, Germany ⁴Working Group for Airway Pharmacology, Fraunhofer Institute for Toxicology and Experimental Medicine Hannover, Germany

For correspondence please contact:

Prof. Dr. Gesine Hansen

Department of Pediatric Pneumology, Allergology and Neonatology

Hannover Medical School

Carl-Neuberg-Str.1

D-30625 Hannover

Phone: +49 511-532-3220

E-Mail: hansen.gesine@mh-hannover.de

ABSTRACT

Background: Allergic asthma is a chronic lung disease resulting from inappropriate immune responses to environmental antigens. Early tolerance induction is an attractive approach for primary prevention of asthma.

Objective: We analysed the mechanisms of perinatal tolerance induction to allergens with particular focus on the role of B cells in preconceptional and early intrauterine immune priming.

Methods: Wild type (WT) and B cell deficient mice received ovalbumin (OVA) intranasally before mating. Their offspring was analysed in a murine model of allergic airway inflammation.

Results: While antigen application before conception protected WT progeny from allergy, it aggravated allergic airway inflammation in B cell deficient offspring. B cell transfer restored protection, demonstrating the crucial role of B cells in perinatal tolerance induction. Effective diaplacental allergen transfer was detectable in pregnant WT mice but not in pregnant B cell KO dams, and antigen concentrations in WT amniotic fluid were higher than in IgG-free amniotic fluid of B cell deficient dams. Application of OVA/IgG immune complexes (IC) during pregnancy boosted OVA uptake by fetal dendritic cells (DCs). Fetal DCs in humans and mice expressed strikingly higher levels of Fc γ receptors compared to DCs from adults and were highly efficient in taking up OVA-IC. Moreover, murine fetal DCs effectively primed antigen-specific foxp3⁺ Tregs after *in vitro* coincubation with OVA/IgG containing amniotic fluid.

Conclusion: Our data support a decisive role for B cells and immunoglobulins during *in utero* tolerance priming. These findings improve the understanding of perinatal immunity and may support the development of effective primary prevention strategies for allergy and asthma in the future.

Key messages

- Mucosal antigen application to female mice before conception protects WT progeny from experimental allergic airway inflammation.
- In absence of B cells, the same intervention aggravates the allergic phenotype in the offspring.
- Antigen/IgG containing amniotic fluid from preconceptionally tolerized dams primes antigen-specific foxp3⁺ Tregs.
- Fetal dendritic cells are highly efficient in taking up immune complexes in both humans and mice.

Capsule Summary

Maternal allergen application before conception protects murine WT-offspring from allergy but aggravates allergic airway inflammation in B-cell deficient pups. Perinatal tolerance induction is associated with intrauterine antigen/IgG-transfer to fetal DCs which in turn effectively prime antigen-specific Tregs.

Key words

Allergy, Asthma, Tolerance, Perinatal, Prenatal, B cells, Tregs, Immunoglobulins, Immune Complexes, Tolerance, Amniotic Fluid

Abbreviations

AF:	amniotic fluid
BALF:	bronchoalveolar lavage fluid
BLG:	beta lactoglobulin
DC:	dendritic cells
FcγR:	fragment crystalizing gamma receptor
FcRn:	neonatal Fc Receptor
fluoOVA:	fluorescence labelled ovalbumin
F1:	first offspring generation
IC:	immune complex
Ig:	immunoglobulin
IL:	interleukin
μMT:	B cell deficient mice
OVA:	ovalbumin
OVA-IC:	OVA/IgG immune complexes
Th:	T helper
Tregs:	regulatory T cells
WT:	wild type

Introduction

More than 300 million people worldwide suffer from allergic asthma, a multifactorial disorder influenced by a multitude of genetic and environmental factors [1-3]. Amongst paediatric patients, it is one of the leading causes of morbidity, affecting around 10% of children in the Western world [4, 5]. Although pharmacotherapy can effectively control asthma symptoms, no curative or effective prevention strategies exist. A number of epidemiological studies demonstrated the protective impact of environmental factors in early childhood (e.g. farm living or attending day care centres) on a child's asthma risk [3, 6-9]. Moreover, maternal factors during pregnancy and lactation such as microbiota, allergen exposure, stress, air pollution, and various others were shown to influence an individual's allergic susceptibility [10-14].

Mucosal exposure towards allergens can induce antigen-specific tolerance [15], and the development of tolerance against allergens can already start during prenatal life. In a murine model, we and others previously demonstrated that antigenic exposure of the mother even before pregnancy crucially influences the progeny's allergy risk [16-18]. In our study, tolerization of female mice before mating induced a long-lasting and antigen-specific protection from the allergic phenotype in the offspring which depended on transfer of antigen-specific immunoglobulins (Ig), transferred either *in utero* or via breast milk after birth [17].

Later it was shown that maternofetal tolerance priming continues into the postnatal period through breast milk mediated transfer of IgG/allergen complexes which induce antigen-specific Tregs [16, 19]. As it is known that antigen transfer into the amniotic fluid can already shape the fetal immune response [20], we were interested in further elucidating the very early steps of perinatal tolerance priming.

The aim of the present study was to analyze by which mechanisms the mother's preconceptional allergen experience influences the offspring's allergic susceptibility. Specifically, we wanted to analyze to what extent maternofetal allergy prevention to harmless antigens, such as allergens acquired in female subjects before pregnancy, is influenced by B cells.

To this end, we analysed the influence of preconceptional allergen application in wild type (WT) and B cell deficient mice. We employed B cell deficient μ MT mice on a C57Bl/6 background which lack the immunoglobulin μ chain gene and have no mature B cells [21, 22]. Murine mothers received antigen before mating, and the allergic phenotype as well as intrauterine antigen and immunoglobulin (Ig) targeting was analysed in their offspring. Furthermore, the tolerogenic potency of amniotic fluid from antigen exposed dams and the antigen binding and Treg inducing capacity of fetal and adult dendritic cells (DC) were assessed.

Whilst maternal antigen exposure protected WT pups from allergy, it aggravated allergic airway inflammation in B cell deficient mice. The lack of B cells was associated with reduced maternofetal Treg priming during lactation, and a marked impairment of intrauterine allergen uptake by fetal DCs.

Moreover, amniotic fluid-induced Treg priming after OVA exposure of pregnant dams was significantly reduced in B cell deficient mice.

Intrauterine allergen transfer and uptake by fetal DCs, as well as antigen specific *in vitro* Treg priming were restored after application of OVA complexed to antigen-specific IgG.

Taken together, our results support a decisive role for B cells and IgG during *in utero* priming of immunological tolerance and may contribute to the development of potent prevention strategies for allergy and asthma.

Material and Methods

Mice

B cell deficient B6.129S2-Ighm^{tm1}Cgn /J (μMT) and wild-type C57BL/6J (WT) mice were obtained from Jackson (Bar Harbor/USA). Ovalbumin (OVA) specific T cell receptor transgenic mice (C57BL/6-Tg(TcraTcrb)425Cbn/J;OT-II CD45.1) were provided by Oliver Pabst, Hannover Medical School. All experiments were approved by local authorities (LAVES#10/0300). Mice were age/gender matched between groups and housed under specific pathogen free conditions.

Maternal antigen administration and murine model of allergic airway inflammation

Murine mothers were anesthetized with isofluran and received 500 μg OVA (grade V; Sigma-Aldrich, Hilden/Germany, product #A5505, Lot # 038K7012) intranasally (i.n.) on day -6 and -3 before mating. Pups were tested in the allergic airway inflammation model with OVA (grade V) or beta lactoglobulin (BLG Sigma-Aldrich, Hilden/Germany, product #L2506, Lot # 096K7009). Mice were intraperitoneally sensitized with OVA (20 μg i.p.) adsorbed to aluminium-hydroxide (alum; Thermo-Fisher, Waltham/USA) followed by i.n challenges (20 μg) as described in Fig. 1. For testing of tolerance induction as shown in Fig. S1, tolerization and induction of allergic airway inflammation was conducted in 8-10 week old B cell deficient mice, and LPS-free OVA (polymixin-based LPS clearance of grade V Sigma OVA) was used.

B cell transfer

For transfer of B cells, CD19⁺ cells were isolated from naïve C57Bl/6 WT mice by magnetic cell sorting (MACS mouse B cell isolation kit, Miltenyi-Biotec, Bergisch-Gladbach/Germany). 2x10⁷ WT B cells were injected intravenously (i.v.) to the tail vein of female μMT mice 10 days before mating.

Measurement of lung function

Invasive lung function was assessed as described previously [23]. In brief, mice were anesthetized and airway hyperreactivity was assessed under increasing doses of methacholine [24].

Analysis of Bronchoalveolar Lavage Fluid (BALF) and lung histology

BALF, cytopins and lung histologies were performed as described before [25]. Histologies were photographed with an AxioCam-ICc1 camera and further analysed with AxioVision/V4.8.2 (Zeiss, Jena/Germany) and in house programs [25]

Determination of antigen-specific Ig levels

OVA specific IgG1/IgE were determined by ELISA as previously described [25].

ELISPOT assay

Total and OVA specific IgM antibody secreting cells were assessed after overnight culture on precoated MultiScreen HTS-IP plates (Millipore, Schwalbach/Germany), followed by incubation with anti-mouse IgG-HRP (SouthernBiotech, Birmingham/USA) and AEC substrate (Vector Laboratories, Peterborough/United Kingdom). Spots were scanned by Eli.Scan F3200 (A.EL.VIS GmbH, Hannover/Germany).

Cytokine measurements

Antigen-specific restimulation was performed as previously described [25]. Cytokines were measured with specific ELISAs (R&D systems, Minneapolis/USA) or cytometric bead arrays (Biolegend, San Diego, USA or Affymetrix, Santa Clara, USA) according to the manufacturer's recommendations.

Collection of amniotic fluid, IC administration and *in vivo* antigen-tracking

Amniotic fluid (AF) was collected as previously described [26]. On pregnancy day 19/20 (matched experimental groups in each experiment), AF was collected from naïve mice or preconceptionally OVA-treated mice (2x500µg i.n. on d-6 and -3 before mating and 40mg OVA i.v. 6 hours prior to sacrificing). Samples were spun down (2000xg, 10 min), then supernatants were immediately frozen. For tracking of DC antigen uptake, pregnant mice received 400µg of Alexa flour labelled OVA i.v. (AF647 or AF488 fluoOVA, Life-Technologies, Carlsbad/USA) or OVA immune complexes (IC) manufactured by incubating 400µg fluoOVA with 200µg OVA specific mouse IgG1 (clone L71, Chondrex, Redmond/USA) at 37°C for 30 min, 6 hours later, mothers were sacrificed and fetal organs were dissected, digested with collagenase/DNase for 20 min at 37°C, erylsed and analysed.

Antigen and Immune complex uptake *in vitro* by murine and human DCs

Murine fetal and adult splenocytes were co-incubated with fluorescence-labelled OVA (100µg/ml), or fluorescence-labelled OVA-IC (100µg/ml fluoOVA plus 200µg/ml OVA specific IgG1 clone L71 preincubated 30 min at 37°C) at 37°C for 10 minutes. Cells were washed three times, and analysed by flow cytometry. All human cord blood experiments were approved by the local ethics committee, and all donors gave their informed consent. Mononuclear cells from peripheral or cord blood were isolated by density centrifugation from healthy donors and placental blood from healthy pregnancies. All cord blood donating mothers were non-atopic, two donors of adult peripheral blood were atopic and suffered from allergic rhinitis and atopic dermatitis, respectively (donor specific data included in suppl. Fig. 6). Mononuclear cells were exposed to OVA (25µg/ml) or OVA-IC (25µg/ml fluo-labelled OVA and 25µg/ml rabbit IgG (ICN-Biomedicals, Irvine/USA) preincubated 30 min at 37°C) for 10 minutes, washed three times and further analysed (HLA-DR (G46-6), CD11c (S-HCL-3), lin-cocktail 2 (Becton-Dickinson, Franklin Lakes/USA).

Western blot of amniotic fluid

AF was mixed with loading buffer (BioRad, Munich/Germany), boiled for 10 min at 95°C and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (7.5% AnykD-gel, BioRad, Munich/Germany) with consecutive nitrocellulose membrane transfer. Within each experiment, same amounts of AF from each group were loaded (50-150µg, same amount of protein/lane on each gel). OVA was detected with rabbit anti-OVA (Ab1221, Abcam, Cambridge/UK), followed by HRP-conjugated goat anti-mouse IgG1 (Jackson-ImmunoResearch, Suffolk/UK). Signals were captured using enhanced chemiluminescence (Thermo-Scientific, Rockford/USA) in a luminescence-imager (DNR Bio-Imaging, Jerusalem/Israel, Chemidic, BioRad, Munich/Germany).

Antigen-specific T cell priming

OVA specific spleen and lymph node CD4 T cells were purified from OT-II/CD45.1 mice by MACS (mouse CD4 T cell isolation kit, Miltenyi-Biotec, Bergisch-Gladbach/Germany). For *in vitro* experiments, T cells were co-incubated with adult or fetal DCs isolated by MACS (CD11c⁺, Miltenyi-Biotec, Bergisch-Gladbach/Germany) or FACS (CD11c⁺/CD11b⁺ cells) in a ratio of 1:50, and with amniotic fluid (dilution 1:50 - 1:10, matched between groups within each experiment), or 100µg/ml OVA, or OVA-IC (100µg/ml OVA plus 200µg/ml OVA IgG1 clone L71 preincubated for 30 min at 37°C) for five days. For *in vivo* experiments, one-day-old pups received 5x10⁶ OT-II/CD45.1 CD4 T cells subcutaneously with simultaneous application of 500µg OVA i.n. to their preconceptionally tolerized mothers. Two weeks later, OT-II/CD45.1⁺CD4⁺ spleen and lymph node T cell populations of 500-5000 cells were detectable in more than 75% of both WT and deficient pups which were then used for further analyses. T cell priming was analysed using CD4 (Becton-Dickinson, Franklin Lakes/USA), Vbeta5.1/5.2-TCR (Clone MR9-4), CD45.1, CD25, and foxp3 (eBioscience, San Diego/USA) with a FACS-Canto II (Becton-Dickinson, Franklin Lakes/USA) and FlowJo V10 (TreeStar, Ashland/USA).

Statistics

Graphs were created with GraphPad Prism5 (GraphPad-Software Inc., La Jolla/USA). Depending on data structure which was assessed by Kolmogorov-Smirnov normality testing, Student's t-test or Mann-Whitney-test (two experimental groups) or one-way-ANOVA/Tukey or Kruskal-Wallis/Dunn's-testing (three groups) were applied. Paired Ttesting or Wilcoxon-matched-pair-testing was applied to analyse adult vs. fetal/cord IC uptake or paired cytokine stimulations from more than one experiment.

RESULTS

Allergic airway inflammation is inhibited by preconceptional mucosal antigen application in the offspring of WT mice while the same protocol increases airway inflammation in B cell deficient mice, which is partly restored by B cell transfer.

To study the impact of B cells in the context of maternal preconceptional antigen exposure on the offspring's allergy risk, female WT and B cell deficient mice received two doses of the model allergen OVA mucosally in the week before mating and the progeny of these two different mouse strains was tested in a murine model of allergic airway inflammation (Fig. 1). In B cell deficient μ MT mice [21], a targeted disruption in the gene encoding for Ig μ leads to a lack of mature B cells and Ig. In spite of their B cell deficiency with resulting lack of CD19⁺CD5⁺ regulatory B cells (Suppl. Fig. 1a) and impaired T cell priming [27], B cell deficient mice develop an asthma-like phenotype and can be successfully tolerized by mucosal antigen application (Suppl. Fig. 1 and [22, 28, 29]).

In the progeny of WT mice, preconceptional mucosal antigen administration protected the offspring from allergic airway inflammation as shown by reduced leukocytic infiltrates in H&E stained lung sections (Fig. 2a,b) as well as reduced total cell and eosinophil numbers in the BALF (Fig. 2c). In contrast, preconceptional antigen application to B cell deficient female mice did not protect the progeny from developing an allergic phenotype, but aggravated their allergic airway inflammation. Leukocytic infiltrates in lung sections as well as total cell numbers and eosinophil numbers in the BALF were significantly increased compared to the offspring of B cell deficient mice that did not receive OVA mucosally before mating (Fig. 2d, e).

The B cell and Ig repertoire in B cell deficient mice can be partially restored by adoptive transfer of WT B cells [27]. To examine whether B cell transfer to the mother abolished the pro-allergenic effect of preconceptional antigen administration, WT B cells were transferred to B cell deficient female mice before mucosal OVA application and consecutive mating. The transferred cells engrafted in recipient mice, as shown by enlarged spleens, detection of B220⁺/IgD⁺ cells in the bone marrow of transplanted mice as well as OVA specific IgM and IgG production (Suppl. Fig. 4).

Indeed, B cell transfer before mucosal antigen application and mating of B cell deficient mice reduced the allergic airway inflammation in the offspring compared to that of pups from mothers that were treated comparably but did not receive B cells. The offspring of B cell transplanted dams displayed a significantly reduced eosinophilic airway inflammation in H&E stained lung sections and lower total and eosinophil BALF cell counts (Fig. 2g-i).

Mucosal antigen application to female mice before mating results in inhibition of IgE and Th2 cytokine production in WT mice but aggravates Th2 cytokines in B cell deficient mice; this effect is restored by B cell transfer.

In WT mice, mucosal antigen application before mating resulted in significantly reduced serum titres of antigen-specific IgE and IgG1 in the offspring (Fig. 3a,b), again demonstrating the maternofetal

transfer of tolerance and allergy protection [17, 18]. As expected, antigen specific IgE and IgG1 were not detectable in B cell deficient mice (Fig. 3 d, e), also not in offspring mice around 12 weeks after B cell transfer to the B cell knock out mother (Fig. 3f).

In WT mice, perinatal tolerance transfer was again demonstrated by significantly reduced antigen-specific Th2 cytokines in cell culture supernatants of splenocytes and bronchial lymph node cells after restimulation with OVA (Fig. 3c-d). In contrast, in the same setting, Th2 cytokines were significantly increased in the offspring of B cell deficient mice that had received OVA mucosally before mating (Fig. 3e). As seen for airway inflammation, reconstitution of B cell deficient mice by B cell transfer reversed this increase and led to significantly reduced Th2 cytokines.

Our data show that mucosal application of the model allergen OVA to female mice before mating protects the offspring from the development of allergic airway inflammation while it aggravates allergic airway inflammation in B cell deficient mice. Transfer of B cells to B cell deficient mice before mucosal antigen application leads to protection of the offspring as seen in WT mice demonstrating the critical role of B cells for the observed effect.

To examine whether the pro-allergenic effect of maternal tolerization in B cell deficient mice was antigen-specific, female B cell deficient mice received OVA before mating and after weaning, while their progeny was tested in a model of allergic airway inflammation based on the unrelated antigen beta lactoglobulin (BLG; Suppl. Fig. 3a). When immunized with BLG, the maternal intervention induced no significant changes of allergic symptoms in the progeny (Suppl. Fig. 3b-d) confirming antigen-specificity of maternal immune priming which had previously been shown by us and others for maternofetal tolerance induction in WT mice [16-18].

In summary, mucosal antigen application before conception reduced allergic symptoms in the WT offspring while it aggravated allergic airway inflammation in the B cell deficient progeny in an antigen-specific manner. B cells were crucial for perinatal tolerance transfer and protection since reconstitution of B cell deficient mice by B cell transfer resulted in abrogation of the proallergenic effect in their offspring.

Impaired Treg priming and intrauterine OVA transfer in B cell deficient mice

A central mechanism in maternofetal tolerance priming is the induction of antigen-specific Tregs [16, 19]. B cell deficient μ MT mice are known to have disturbed Treg expansion [27, 30], and indeed we observed reduced frequencies of Tregs in two-week-old μ MT pups (Fig. 4a).

To test whether this Treg deficiency also affected maternofetal priming of antigen-specific Tregs, we transferred antigen-specific CD4⁺ T cells from OVA-T cell receptor transgenic OT-II mice to one-day-old WT and B cell deficient pups of naïve and OVA tolerized murine mothers. Two weeks later, the frequency of foxp3⁺ Tregs within the transferred OVA specific CD4⁺ T cell population in spleens and lymph nodes of recipient pups was analysed. A significantly higher rate of antigen-specific Tregs was observed in the WT compared to B cell deficient offspring (Fig. 4b), illustrating a marked deficiency in maternofetal antigen-specific Treg priming in B cell deficient mice.

To explore by which mechanism prenatal maternal antigen exposure modulated the pups' immune response and how the observed marked deficiency in maternofetal Treg priming may influence previous observations in our allergic airway inflammation model, we next investigated the amount, fate, and tolerogenic potential of intrauterinely transferred antigen in WT and B cell deficient mice. Because we could not track OVA in the amniotic fluid or on fetal DCs after preconceptional mucosal antigen administration alone, preconceptionally OVA treated murine mothers received an additional high dose fluorescently labeled antigen (fluoOVA) intravenously shortly before analyses of intrauterine transfer and fetal DC tracking.

When we compared the *in utero* targeting of maternally administered antigen, higher proportions of fluoOVA⁺ fetal splenic and thymic DCs were observed in the fetuses of OVA exposed WT mothers compared to those of OVA exposed B cell deficient mothers (Fig. 4c). Moreover, transfer of i.v.-administered OVA to the amniotic fluid (AF) was far more efficient in WT mothers, and - as expected - intrauterine OVA-specific IgG was only observed in tolerized WT but not in B cell deficient mothers (Fig. 4d,e and suppl. Fig. S5).

Next, we were interested to study the implications of this differential amniotic fluid composition in WT versus B cell deficient mice with regard to antigen-specific tolerance and Treg priming in the offspring. For this purpose, we isolated T cells from OT-II donor mice that are transgenic for an OVA-specific T cell receptor. Moreover, we isolated DCs from WT fetal mice and cocultured them with OVA specific T cells and the amniotic fluid of pregnant WT or B cell deficient mice which were either naïve or OVA-tolerized. In this coculture, AF from WT mothers induced a significant increase of antigen-specific CD4⁺CD25^{hi}foxp3⁺ Tregs, while the ratio of CD4⁺CD25^{hi}foxp3⁻ effector T cells to CD4⁺CD25^{hi}foxp3⁺ Tregs was significantly reduced. In contrast, AF from B cell deficient mothers was not as effective in priming a tolerogenic T cell response (Fig. 4f-h).

Transplacental antigen targeting to FcγR^{hi} DCs is augmented by immune complexing

To further understand the interplay of antigen, immunoglobulins and DCs during intrauterine immune priming, we next compared the phenotype and function of fetal and adult DCs. In both WT and B cell deficient mice, fetal DCs expressed the Fcγ receptor CD16/32 significantly stronger than their adult counterparts (Fig. 5a,b). CD16/32 binds immune complexes [31], and OVA-IC uptake was significantly stronger in fetal than in adult DCs with the strongest uptake in CD16/32 Fcγ receptor^{hi} fetal DCs (Fig. 5 c,d). To test whether this highly efficient IC-uptake by fetal DCs was also relevant *in vivo*, preconceptionally OVA exposed pregnant B cell deficient mothers received fluoOVA or IgG/fluoOVA immune complexes (IC) intravenously and the antigen uptake of fetal DCs was analysed. Indeed, also *in vivo*, IgG-complexing of OVA significantly increased the antigen uptake in the B cell deficient fetal DCs (Fig. 5e,f).

Next, we tested the relevance of IC-augmented antigen uptake in DCs derived from human cord blood and human peripheral blood from adults. Also in the human setting, OVA-uptake was significantly

boosted by immune complexing in DCs from both adults and cord blood donors. (Fig. 5g), and the ratio of OVA positive DCs after OVA-IC vs OVA coincubation alone was significantly higher in cord blood samples (Fig. 5h and suppl. Fig. 6). These observations point to a central role of immune complexing in fetal DC antigen uptake also in man.

Discussion

Our data demonstrate the strong impact of B cells in perinatal tolerance priming. We show that preconceptional maternal antigen exposure induces antigen-specific tolerance in the WT offspring, but aggravates allergic airway inflammation in the progeny of B cell deficient mice. B cell deficient mice show a marked impairment of maternofetal Treg priming during lactation and a significantly reduced intrauterine antigen transfer and uptake by fetal DCs. Both WT and B cell deficient fetal DCs express strikingly high levels of Fc γ R and are highly efficient in taking up IgG-complexed OVA. Moreover, we found fetal DCs to induce antigen-specific Tregs when coincubated with OVA/IgG containing amniotic fluid of WT dams but not when coincubated with amniotic fluid of OVA exposed B cell deficient mice.

The observed intrauterine antigen transfer in WT mice and its potency in immunological priming support previous findings on the critical role of intrauterine OVA transfer in WT mice. Utthof *et al.* showed that *in utero* transferred maternal antibodies inhibit the offspring's T cell antigen-specific responses [26]. In addition, we show that this process is critically regulated by IgG and significantly impaired in the absence of B cells. Moreover, we present the novel finding that AF from tolerized WT mothers primes Tregs, an observation so far only described for IC containing breastmilk of antigen exposed dams [16]. Our experiments demonstrate that IgG is central in promoting intrauterine antigen transfer, antigen targeting to fetal DCs, and priming of antigen-specific Tregs. Although our experimental setup may be unphysiological to the point that we used mature antigen specific T cells to assess potential priming effects of amniotic fluid - whilst T cell maturation in mice is not completed *in utero* - our results still point to a decisive role of B cells and antigen specific IgG in the early shaping of T cell responses. This notion is further supported by recent work by Gupta *et al.*, who showed effective tolerance induction in a mouse model of haemophilia. In their model, intrauterine transfer of IgG Fc-fused Factor VIII lead to effective intrauterine DC targeting of this protein with consecutive promotion of Tregs in the pups which were protected from antigen specific alloimmunization in later life [32].

One of our most surprising findings was the antigen-specific proallergic effect of maternal antigen application in B cell deficient mice. Our results suggest that in the absence of B cells, low doses of antigen are transferred *in utero* and induce a pro-allergic, T effector cell biased immune response. This observation fits to the previously described deficiency of B cell deficient mice in low antigen dose induced oral tolerance [33] and the strong dose dependency of mucosal tolerance development [34]. Importantly, our findings expand previous knowledge on the immune priming effect of maternal IC containing body fluids, which had primarily focused on breast milk, to the prenatal period [16, 19, 35-38] and go in line with our previous finding that maternofetally induced tolerance can be achieved in WT pups even if the offspring is not nursed by their allergen exposed mother but by an antigen naïve wet nurse [17]. We chose our previously described approach of preconceptional application of high antigen doses, because this protocol had shown to induce tolerance in WT offspring mice and it

ensured induction of OVA specific IgG before and during early pregnancy [17, 18]. Our own work and that of many others support the notion that the perinatal phase (including pregnancy and lactation) represents a favourable time window to induce profound antigen specific tolerance [16-19, 32, 39]. Because our main interest was to analyze how the mother's preconceptional exposure to harmless antigens influences the allergy risk in the offspring, we chose to administer high doses of mucosal antigen to the mother before mating, similar to the approach previously described by our group [17, 18]. For our main *in vivo* experiments, we chose the intranasal route of antigen administration as this approach had been demonstrated to be highly effective in intragenerational tolerance induction [29, 40] as well as transmaternal tolerization [16, 19]. Although the proallergenic effect of maternal ovalbumin administration varied from experiment to experiment, B cell deficient offspring mice consistently showed no or inversed effects of maternal tolerization compared to WT offspring mice in the allergic airway inflammation model. Previously, we had described that preconceptionally induced maternofetal tolerance priming depended on the transfer of IgG and did not occur in FcRn deficient mice lacking the neonatal receptor for diaplacental IgG transfer [17, 41]. However, FcRn is also important in shuffling albumin proteins via the placenta [42]. Hence, intrauterine transfer of the model allergen ovalbumin was also hindered in FcRn deficient mice which completely prevented prenatal antigen-specific T cell priming in this strain [17, 32]. By contrast, through specific knockout and re-introduction of B cells and IgG, our current *in vivo* and *in vitro* experiments allowed for the comparison of specific immunological effects of free OVA versus OVA/IgG-IC in the perinatal setting.

Our findings on intrauterine antigen/IgG transfer may also be relevant in humans. It has been shown that common asthma allergens as well as allergen specific IgG can be delivered transplacentally to the human fetus [43-48]. For infectious antigens such as plasmodium proteins, it was demonstrated that human diaplacental antigen transfer is facilitated when these proteins are bound to specific IgG and transferred in IC form [49]. Interestingly, IgG transfused to human mothers, for example in pregnant women receiving intravenous Ig, is effectively transferred to the offspring via placenta and breast milk [50].

Another striking finding in our study that supports a central role for Ig-fusion in intrauterine antigen trafficking was the highly significant increase in FcγR expression on murine fetal DCs and the enhanced OVA-IC uptake in fetal than in adult DCs. Also in human adult and cord blood DCs, antigen uptake was significantly boosted by immune complexing. Together with our *in vitro* data and recent findings by others on Treg induction after intrauterine Fc-fused antigen exposure [32], this suggests that fetal DCs are remarkably efficient in taking up IC and priming tolerogenic T cell responses. Of note, also other Fc receptors, especially FcRn, are upregulated in murine perinatal APCs [51, 52] and we cannot exclude that these may have also contributed to the enhanced IC uptake by fetal DCs observed in our model.

One of the immediate effects of IC binding is accumulation of antigen on the cell surface, which is associated with DC maturation and enhanced antigen internalization and presentation [31, 53] leading to modulation or suppression of immune responses in mice and man [19, 54-57]. More than 15 years ago, Machiels and colleagues treated asthma patients by intradermal inoculation with IC manufactured from allergen extract and excess autologous IgG. They observed a remarkable treatment response, and a similar IC therapy success was later observed in patients with allergic rhinitis [57, 58].

Although obvious differences between murine and human immune ontology exist, gestation generally appears to be a favourable time window to prime immunological tolerance in humans as well [17, 32, 59, 60]. A recent paper demonstrated that already in human cord blood, around 1-3% of the CD4⁺ T cells display a memory phenotype [61]. Children born in the farming environment, which is known to protect from allergies, display already at birth higher rates of Tregs and a tolerogenic cytokine response upon leukocyte stimulation [62, 63]. However, although it is still unclear by which exact mechanisms and to what extent this early priming affects the development of allergies in later life, these findings suggest that a tolerogenic T cell memory protecting the child from allergies can be primed prenatally in humans too [64].

To further understand the relevance of our findings, future work will focus on analysing the exact composition and modification of murine and human antigen-specific Ig and IC that are transferred *in utero*, and on their nature in healthy human pregnancies and mothers and neonates with allergic predisposition. On the long run, this may contribute to an improved understanding of perinatal immunity and the development of potent prevention strategies for allergy and asthma.

In conclusion, our current work supports a decisive role for B cells and immunoglobulins during *in utero* tolerance priming. It shows that one mechanism by which maternal antigen experience shapes the offspring's immune response is the intrauterine transfer of IC. This appears to be a protective mechanism, introducing the neonatal immune system to antigens and defining Treg-worthy friend or T effector cell requiring foe. Our data improve the understanding of perinatal immune priming and may support the development of effective primary prevention strategies in asthma in the future.

Fig. 1 Experimental scheme for evaluating the effects of maternal tolerization on the offspring's allergy risk.

Murine mothers (WT or B cell knockout) received two mucosal OVA doses and were consecutively mated. After weaning, offspring mice were tested in the OVA based allergic airway inflammation model. In some experiments, B cell deficient mothers received WT B cells by tail vein injection three days before the first OVA dose.

Fig. 2 Preconceptional mucosal antigen application to the dam reduces allergic airway inflammation in the offspring but aggravates airway inflammation in B cell KO mice; this is abolished after transfer of B cells to B cell deficient mothers before antigen administration.

Offspring of tolerized WT mothers display (a) reduced lung inflammation and mucus production in H&E and PAS stained paraffin-embedded lung-slices, (b) reduced airway inflammation in investigator independent analysis of H&E stained lung sections, and (c) reduced total cell numbers and eosinophilia in BALF compared to the offspring of naïve WT dams. In B cell KO offspring, maternal OVA administration leads to (d) aggravated lung inflammation and mucus production and (e) increased airway inflammation in investigator independent analysis of H&E stained lung sections, and (f) increased total numbers and eosinophils in the BALF. B cell transfer to B cell deficient dams before antigen administration leads to (g) reduced lung inflammation and mucus production, (h) reduced airway inflammation in investigator independent analysis of H&E stained lung sections, and (i) reduced total numbers and eosinophils in the BALF compared to offspring of tolerized B cell knockout mothers without B cell transfer.

Alum: control group; OVA: allergic group; M^{ova} OVA: allergic offspring of OVA exposed dams; B cell Tx: allergic offspring of OVA exposed and B cell transplanted dams, eo: eosinophils, mac: macrophages, lym: lymphocytes, neu: neutrophils, WT: $n \geq 6$ mice per experimental group (data from one (a,b) and two representative experiments out of ≥ 3); B cell KO: $n \geq 8$ mice per experimental group (data from one (d,e) and two representative experiments out of ≥ 3); B cell KO+Tx: $n \geq 4$ mice per experimental group (data from one (d,e) and two representative experiments out of ≥ 3), graphs display mean + s.e.m., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Fig. 3 Preconceptional mucosal OVA administration reduces antigen specific immunoglobulins and cytokine production in the WT offspring, while it increases antigen specific cytokine responses in the offspring of B cell deficient mice; this aggravation is partially abolished upon B cell transfer.

Offspring of tolerized WT mice display (a) reduced serum levels of OVA-specific IgE and (b) OVA-specific IgG1, and diminished antigen specific cytokine production from (c) splenocytes and (d) bronchial lymph node cells. Offspring of OVA exposed B cell deficient mothers show (e,f) no immunoglobulin production but (g) increased antigen specific cytokine production by splenocytes and

(h) bronchial lymph node cells. In offspring of B cell transplanted, OVA exposed μ MT mice, (i,j) absent immunoglobulins and reduced antigen specific cytokine production after *in vitro* (k) splenocyte and (l) bronchial lymph node cell restimulation with OVA are observed.

Alum: control group; OVA: allergic group; M^{ova} OVA: allergic offspring of OVA exposed dams; B cell Tx: allergic offspring of OVA exposed and B cell transplanted dam, eo: eosinophils, mac: macrophages, lym: lymphocytes, neu: neutrophils, WT: $n \geq 11$ mice per experimental group from two (a-c) and $n \geq 4$ mice per experimental group from one representative experiment (d), B cell KO: $n \geq 7$ mice per experimental group from two (f,g) and $n \geq 3$ mice per experimental group from one representative experiment (e,h), B cell KO +Tx: $n \geq 7$ mice per experimental group from two (k) and $n \geq 3$ mice per experimental group from one representative experiment (i,j,l), graphs display mean + s.e.m., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Fig. 4 Defective Treg priming and *in utero* antigen transfer in B cell deficient mice

(a) Reduced Treg frequency in two week old B cell deficient pups (frequency of $CD4^+ CD25^{hi} foxp3^+$ T cells, $n \geq 26$ mice per experimental group; data from 3 experiments); (b) defective antigen-specific Treg priming in two week old B cell deficient pups after antigen re-exposure of the mother during lactation (frequency of antigen-specific OTII $CD4^+ foxp3^+$ T cells in recipient pups ($n \geq 6$ mice per group; data from 2 experiments), (c) reduced *in vivo* intrauterine transfer of fluorescently labelled OVA (fluoOVA) to $CD11c/CD11b^+$ fetal dendritic cells in B cell deficient mice (frequency of $OVA^+ CD11c/CD11b^+$ fetal splenic and thymic DCs, means $\pm$ s.e.m. of two experiments each including $n \geq 5$ fetuses per group), (d) OVA specific IgG (ELISA, one representative experiment including $n \geq 5$ fetuses per group) in WT compared to B cell deficient amniotic fluid, (e) transfer of higher amounts of OVA in the amniotic fluid of WT mice that received OVA preconceptionally compared to amniotic fluid of equally treated B cell deficient mice (AF, Western Blot, one representative experiment with pooled amniotic fluid of $n \geq 5$ fetuses per group), (f) priming of antigen-specific $CD4^+ CD25^{hi} foxp3^+$ Tregs by fetal DCs after coincubation with amniotic fluid of naïve WT dams (Ctrl.), OVA exposed WT dams (WT) and OVA exposed B cell deficient mothers (KO), as well as medium alone (Med), OVA immune complexes (IC), and OVA alone. (g) $CD4^+ CD25^{hi} foxp3^-$ T effector to $CD4^+ CD25^{hi} foxp3^+$ Treg ratio in the different coincubation settings (one representative experiment, KO: B cell deficient mice). All bars display mean + s.e.m., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 5 Increased *in vitro* uptake of IC by fetal DCs

(a) Flowcytometry gating and (b) frequency of adult vs. fetal $CD16/32 Fc\gamma R^{hi}$ DCs, (c,d) uptake of fluorescently labelled OVA (fluoOVA) in $CD11b^+ CD11c^+$ fetal and adult DCs after *in vitro* incubation with OVA-IC (percentage of fluoOVA IC^+ DCs in total $CD11b^+ CD11c^+$ DCs, data from ≥ 3 mice/group, one representative experiment), (e,f) uptake of OVA-IC by total fetal DCs after injection

of OVA alone (OVA) or OVA-IC (IC) to B cell deficient pregnant mothers (means $\pm$ s.e.m of 2 experiments each including $n \geq 5$ fetuses per group), (g) antigen uptake in human adult versus cord blood DCs after incubation with OVA or OVA IC and (h) ratio of OVA IC/ OVA antigen uptake from individual donors (data from $n=7$ donors per group, data from 3 experiments). All bars display mean $\pm$ s.e.m., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Acknowledgments

The authors thank Jana Bergmann, Christin Albrecht, Heike Grundmann, and Anika Dreier for their excellent technical support. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 587/TP14), the German Federal Ministry of Education and Research (BREATH/ DZL), Hannover Biomedical Research School (HBRS Molecular Medicine at Hannover Medical School) and the Else Dörenkamp Foundation.

Author contributions

G. Ha., C. Ha., and C. He. conceived the project and designed the experiments. C. Ha., J. S., A. J., A. L. and A. MB. conducted experiments or helped with analysis. H. H. conducted invasive lung function experiments and analysis. C. Ha. and G. Ha. wrote the manuscript. All authors read and approved the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

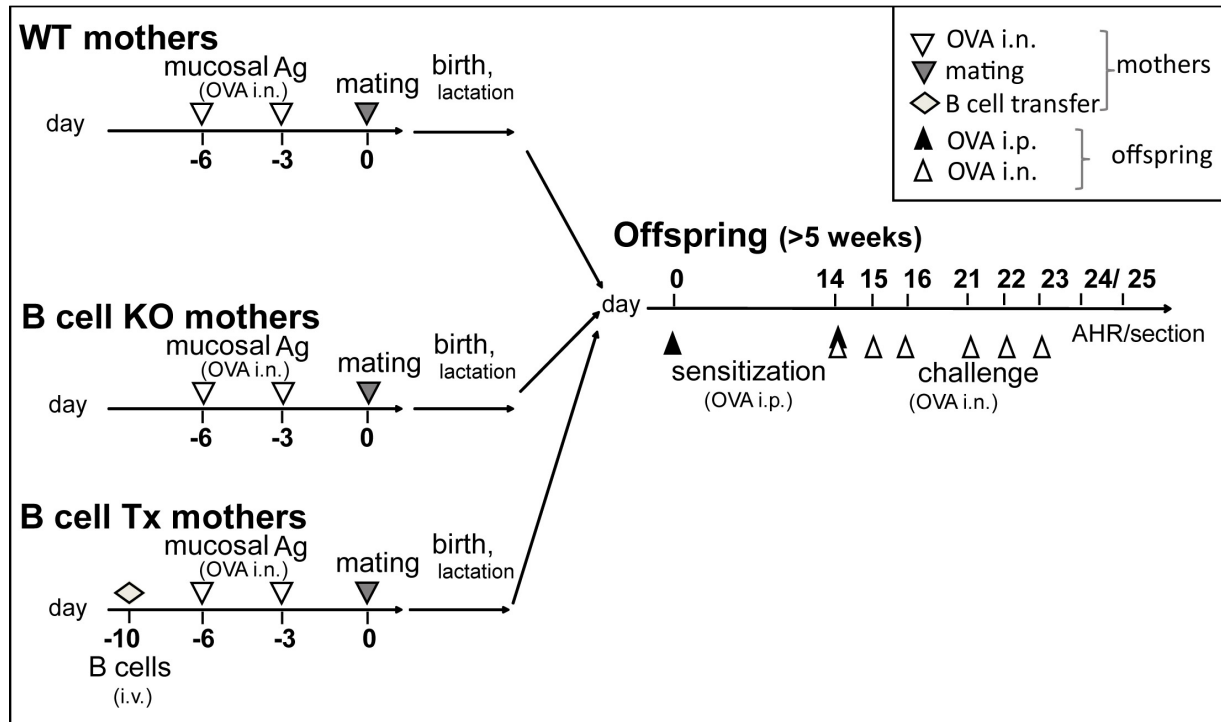
1. Vercelli, D., *Gene-environment interactions: the road less traveled by in asthma genetics*. J Allergy Clin Immunol, 2009. **123**(1): p. 26-7.
2. Spycher, B.D., et al., *Genome-wide prediction of childhood asthma and related phenotypes in a longitudinal birth cohort*. J Allergy Clin Immunol, 2012. **130**(2): p. 503-9 e7.
3. Holt, P.G., et al., *Toward improved prediction of risk for atopy and asthma among preschoolers: a prospective cohort study*. J Allergy Clin Immunol, 2010. **125**(3): p. 653-9, 659 e1-659 e7.
4. Masoli, M., et al., *The global burden of asthma: executive summary of the GINA Dissemination Committee report*. Allergy, 2004. **59**(5): p. 469-78.
5. Bloom, B., L.I. Jones, and G. Freeman, *Summary health statistics for U.S. children: National Health Interview Survey, 2012*. Vital Health Stat 10, 2013(258): p. 1-81.
6. Holt, P.G., et al., *The role of allergy in the development of asthma*. Nature, 1999. **402**(6760 Suppl): p. B12-7.
7. Bach, J.F., *The effect of infections on susceptibility to autoimmune and allergic diseases*. N Engl J Med, 2002. **347**(12): p. 911-20.
8. Ball, T.M., et al., *Siblings, day-care attendance, and the risk of asthma and wheezing during childhood*. N Engl J Med, 2000. **343**(8): p. 538-43.
9. MacNeill, S.J., et al., *Asthma and allergies: is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies*. Allergy, 2013. **68**(6): p. 771-9.
10. Bertelsen, R.J., et al., *Probiotic milk consumption in pregnancy and infancy and subsequent childhood allergic diseases*. J Allergy Clin Immunol, 2013.
11. Maslova, E., et al., *Peanut and tree nut consumption during pregnancy and allergic disease in children-should mothers decrease their intake? Longitudinal evidence from the Danish National Birth Cohort*. J Allergy Clin Immunol, 2012. **130**(3): p. 724-32.
12. Mathilda Chiu, Y.H., et al., *Prenatal and postnatal maternal stress and wheeze in urban children: effect of maternal sensitization*. Am J Respir Crit Care Med, 2012. **186**(2): p. 147-54.
13. Dotterud, C.K., et al., *The impact of pre- and postnatal exposures on allergy related diseases in childhood: a controlled multicentre intervention study in primary health care*. BMC Public Health, 2013. **13**: p. 123.

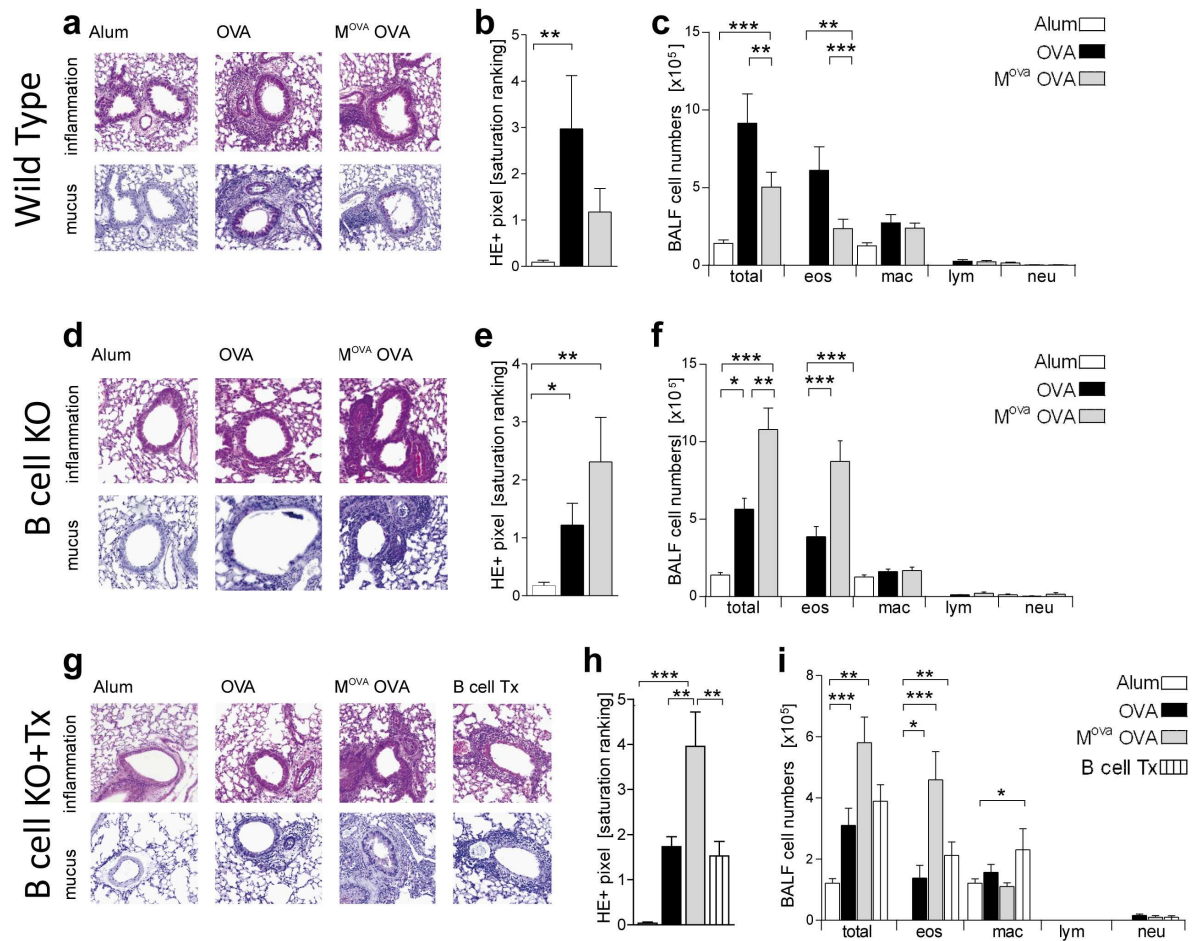
14. Sonnenschein-van der Voort, A.M., et al., *Air pollution, fetal and infant tobacco smoke exposure, and wheezing in preschool children: a population-based prospective birth cohort*. Environ Health, 2012. **11**: p. 91.
15. Kim, S.R., et al., *Inhibition of p38 MAPK reduces expression of vascular endothelial growth factor in allergic airway disease*. J Clin Immunol, 2012. **32**(3): p. 574-86.
16. Mosconi, E., et al., *Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development*. Mucosal Immunol, 2010. **3**(5): p. 461-74.
17. Polte, T., C. Hennig, and G. Hansen, *Allergy prevention starts before conception: maternofetal transfer of tolerance protects against the development of asthma*. J Allergy Clin Immunol, 2008. **122**(5): p. 1022-1030 e5.
18. Polte, T. and G. Hansen, *Maternal tolerance achieved during pregnancy is transferred to the offspring via breast milk and persistently protects the offspring from allergic asthma*. Clin Exp Allergy, 2008. **38**(12): p. 1950-8.
19. Verhasselt, V., et al., *Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma*. Nat Med, 2008. **14**(2): p. 170-5.
20. Blumer, N., P.I. Pfefferle, and H. Renz, *Development of mucosal immune function in the intrauterine and early postnatal environment*. Curr Opin Gastroenterol, 2007. **23**(6): p. 655-60.
21. Kitamura, D., et al., *A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene*. Nature, 1991. **350**(6317): p. 423-6.
22. Jungsuwadee, P., et al., *Repeated aerosol allergen exposure suppresses inflammation in B-cell-deficient mice with established allergic asthma*. Int Arch Allergy Immunol, 2004. **133**(1): p. 40-8.
23. Glaab, T., et al., *Repetitive measurements of pulmonary mechanics to inhaled cholinergic challenge in spontaneously breathing mice*. J Appl Physiol (1985), 2004. **97**(3): p. 1104-11.
24. Hoymann, H.G., *New developments in lung function measurements in rodents*. Exp Toxicol Pathol, 2006. **57 Suppl 2**: p. 5-11.
25. Polte, T., et al., *CD137-mediated immunotherapy for allergic asthma*. J Clin Invest, 2006. **116**(4): p. 1025-36.
26. Uthoff, H., et al., *Critical role of preconceptional immunization for protective and nonpathological specific immunity in murine neonates*. J Immunol, 2003. **171**(7): p. 3485-92.
27. Sun, J.B., et al., *B lymphocytes promote expansion of regulatory T cells in oral tolerance: powerful induction by antigen coupled to cholera toxin B subunit*. J Immunol, 2008. **181**(12): p. 8278-87.

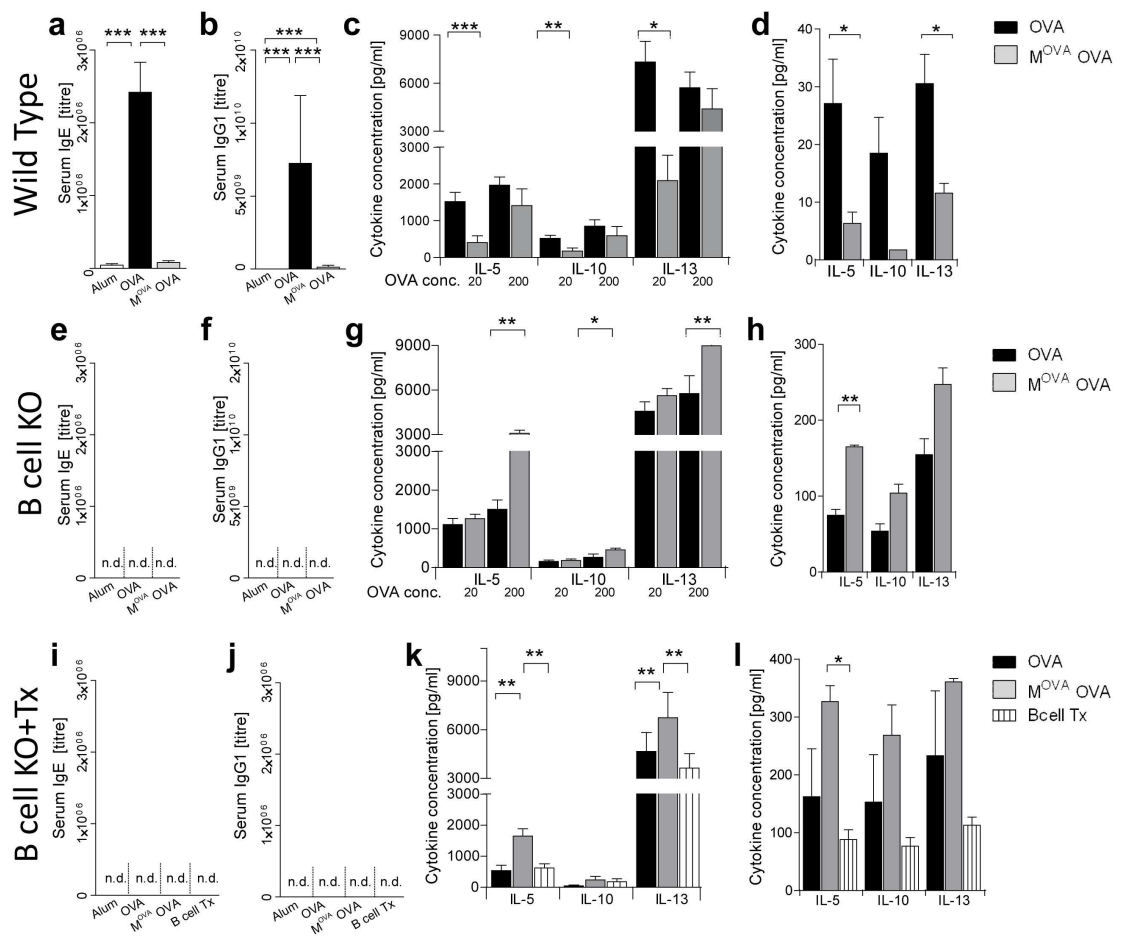
28. Mann, M.K., et al., *B cell regulation of CD4⁺CD25⁺ T regulatory cells and IL-10 via B7 is essential for recovery from experimental autoimmune encephalomyelitis*. J Immunol, 2007. **178**(6): p. 3447-56.
29. Habener, A., et al., *B cell subsets are modulated during allergic airway inflammation but are not required for the development of respiratory tolerance in a murine model*. Eur J Immunol, 2016.
30. Wang, L., et al., *T regulatory cells and B cells cooperate to form a regulatory loop that maintains gut homeostasis and suppresses dextran sulfate sodium-induced colitis*. Mucosal Immunol, 2015. **8**(6): p. 1297-312.
31. Nimmerjahn, F. and J.V. Ravetch, *Fcγ receptors as regulators of immune responses*. Nat Rev Immunol, 2008. **8**(1): p. 34-47.
32. Gupta, N., et al., *Regulation of immune responses to protein therapeutics by transplacental induction of T cell tolerance*. Sci Transl Med, 2015. **7**(275): p. 275ra21.
33. Gonnella, P.A., H.P. Waldner, and H.L. Weiner, *B cell-deficient (mu MT) mice have alterations in the cytokine microenvironment of the gut-associated lymphoid tissue (GALT) and a defect in the low dose mechanism of oral tolerance*. J Immunol, 2001. **166**(7): p. 4456-64.
34. Franco, L., et al., *Priming or tolerization of the B- and Th2-dependent immune response by the oral administration of OVA-DNP is determined by the antigen dosage*. Cell Immunol, 1998. **190**(1): p. 1-11.
35. Leme, A.S., et al., *Role of breast milk in a mouse model of maternal transmission of asthma susceptibility*. J Immunol, 2006. **176**(2): p. 762-9.
36. Matson, A.P., et al., *IgG transmitted from allergic mothers decreases allergic sensitization in breastfed offspring*. Clin Mol Allergy, 2010. **8**: p. 9.
37. Yamamoto, T., et al., *Oral tolerance induced by transfer of food antigens via breast milk of allergic mothers prevents offspring from developing allergic symptoms in a mouse food allergy model*. Clin Dev Immunol, 2012. **2012**: p. 721085.
38. Jarrett, E.E. and E. Hall, *The development of IgE-suppressive immunocompetence in young animals: influence of exposure to antigen in the presence or absence of maternal immunity*. Immunology, 1984. **53**(2): p. 365-73.
39. Alpan, O., G. Rudomen, and P. Matzinger, *The role of dendritic cells, B cells, and M cells in gut-oriented immune responses*. J Immunol, 2001. **166**(8): p. 4843-52.
40. Busse, M., et al., *ICOS mediates the generation and function of CD4⁺CD25⁺Foxp3⁺ regulatory T cells conveying respiratory tolerance*. J Immunol, 2012. **189**(4): p. 1975-82.

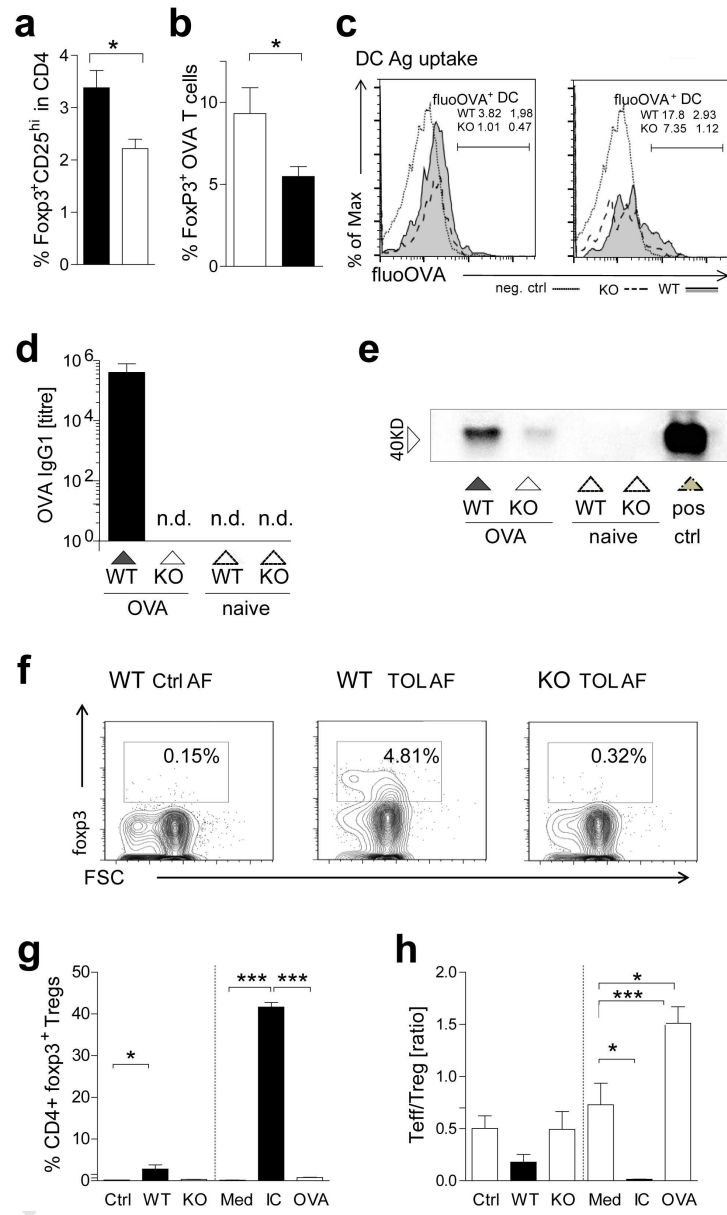
41. Nakata, K., et al., *The transfer of maternal antigen-specific IgG regulates the development of allergic airway inflammation early in life in an FcRn-dependent manner*. *Biochem Biophys Res Commun*, 2010. **395**(2): p. 238-43.
42. Rath, T., et al., *The immunologic functions of the neonatal Fc receptor for IgG*. *J Clin Immunol*, 2013. **33 Suppl 1**: p. S9-17.
43. Macchiaverni, P., et al., *Mother to child transfer of IgG and IgA antibodies against *Dermatophagoides pteronyssinus**. *Scand J Immunol*, 2011. **74**(6): p. 619-27.
44. Loibichler, C., et al., *Materno-fetal passage of nutritive and inhalant allergens across placentas of term and pre-term deliveries perfused in vitro*. *Clin Exp Allergy*, 2002. **32**(11): p. 1546-51.
45. Szepefalusi, Z., et al., *Direct evidence for transplacental allergen transfer*. *Pediatr Res*, 2000. **48**(3): p. 404-7.
46. Edelbauer, M., et al., *Maternally delivered nutritive allergens in cord blood and in placental tissue of term and preterm neonates*. *Clin Exp Allergy*, 2004. **34**(2): p. 189-93.
47. Holloway, J.A., et al., *Detection of house-dust-mite allergen in amniotic fluid and umbilical-cord blood*. *Lancet*, 2000. **356**(9245): p. 1900-2.
48. Vance, G.H., et al., *Exposure of the fetus and infant to hens' egg ovalbumin via the placenta and breast milk in relation to maternal intake of dietary egg*. *Clin Exp Allergy*, 2005. **35**(10): p. 1318-26.
49. May, K., et al., *Antibody-dependent transplacental transfer of malaria blood-stage antigen using a human ex vivo placental perfusion model*. *PLoS One*, 2009. **4**(11): p. e7986.
50. Palmeira, P., et al., *Transfer of antibodies across the placenta and in breast milk from mothers on intravenous immunoglobulin*. *Pediatr Allergy Immunol*, 2009. **20**(6): p. 528-35.
51. Yoshida, M., et al., *Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells*. *Immunity*, 2004. **20**(6): p. 769-83.
52. Liu, X., et al., *The neonatal FcR-mediated presentation of immune-complexed antigen is associated with endosomal and phagosomal pH and antigen stability in macrophages and dendritic cells*. *J Immunol*, 2011. **186**(8): p. 4674-86.
53. Baudino, L., et al., *Differential contribution of three activating IgG Fc receptors (FcγRI, FcγRIII, and FcγRIV) to IgG2a- and IgG2b-induced autoimmune hemolytic anemia in mice*. *J Immunol*, 2008. **180**(3): p. 1948-53.

54. Caulfield, M.J. and D. Shaffer, *Immunoregulation by antigen/antibody complexes. I. Specific immunosuppression induced in vivo with immune complexes formed in antibody excess*. J Immunol, 1987. **138**(11): p. 3680-3.
55. da Costa, P.S., M.S. de Macedo, and A. Perini, *Suppression of mouse IgE response by immune complexes*. J Allergy Clin Immunol, 1990. **86**(4 Pt 1): p. 496-502.
56. Sinclair, N.R., et al., *Regulation of the immune response. X. Antigen-antibody complex inactivation of cells involved in adoptive transfer*. J Immunol, 1974. **113**(5): p. 1493-500.
57. Machiels, J.J., et al., *Allergic bronchial asthma due to Dermatophagoides pteronyssinus hypersensitivity can be efficiently treated by inoculation of allergen-antibody complexes*. J Clin Invest, 1990. **85**(4): p. 1024-35.
58. Machiels, J.J., et al., *Allergen-antibody complexes can efficiently prevent seasonal rhinitis and asthma in grass pollen hypersensitive patients. Allergen-antibody complex immunotherapy*. Allergy, 1991. **46**(5): p. 335-48.
59. Billingham, R.E., L. Brent, and P.B. Medawar, *Actively acquired tolerance of foreign cells*. Nature, 1953. **172**(4379): p. 603-6.
60. Guerau-de-Arellano, M., et al., *Neonatal tolerance revisited: a perinatal window for Aire control of autoimmunity*. J Exp Med, 2009. **206**(6): p. 1245-52.
61. Zhang, X., et al., *CD4 T cells with effector memory phenotype and function develop in the sterile environment of the fetus*. Sci Transl Med, 2014. **6**(238): p. 238ra72.
62. Pfefferle PI, B.G., Blümer N, Roponen M, Ege MJ, Krauss-Etschmann S, Genuneit J, Hyvärinen A, Hirvonen MR, Lauener R, Pekkanen J, Riedler J, Dalphin JC, Brunekeef B, Braun-Fahrländer C, von Mutius E, Renz H; PASTURE Study Group, *Cord blood cytokines are modulated by maternal farming activities and consumption of farm dairy products during pregnancy: the PASTURE Study*. J Allergy Clin Immunol., 2010. **125**(1)(1): p. 108-15.e1-3. .
63. Schaub B, L.J., Höppler S, Schleich I, Huehn J, Olek S, Wiecek G, Illi S, von Mutius E., *Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells*. J Allergy Clin Immunol., 2009. **123**(4): p. 774-82.e5.
64. Renz, H., P. Brandtzaeg, and M. Hornef, *The impact of perinatal immune development on mucosal homeostasis and chronic inflammation*. Nat Rev Immunol, 2012. **12**(1): p. 9-23.









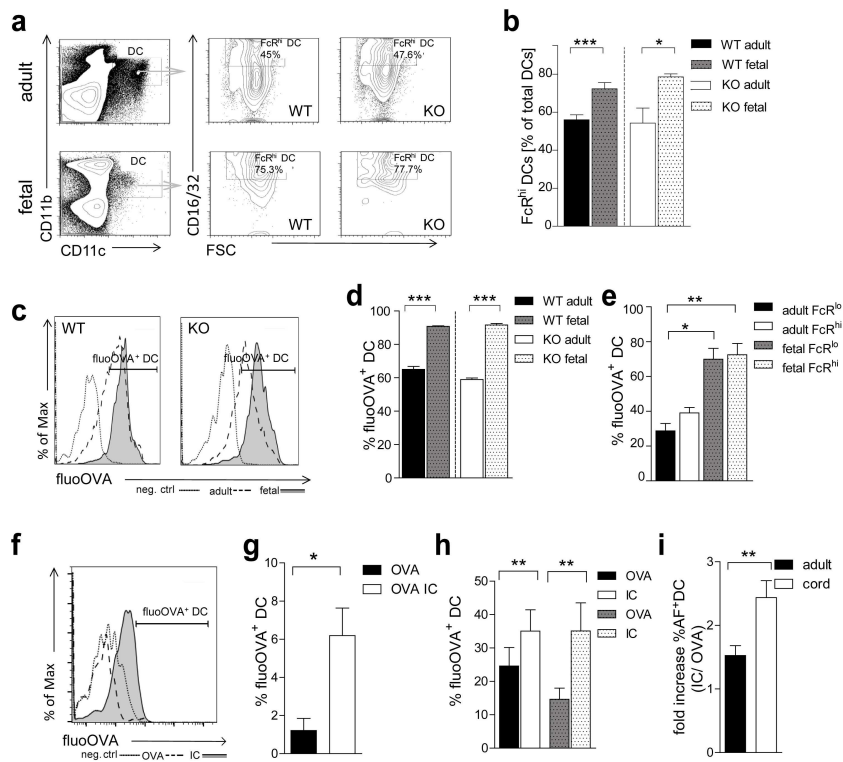


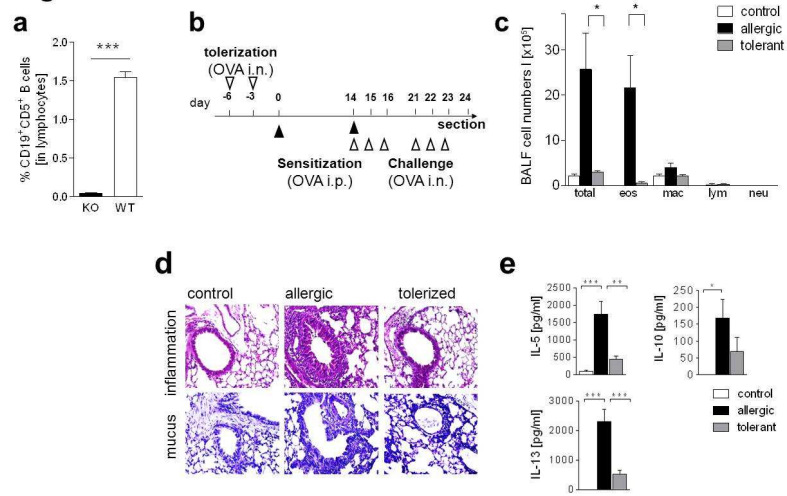
Fig. S1

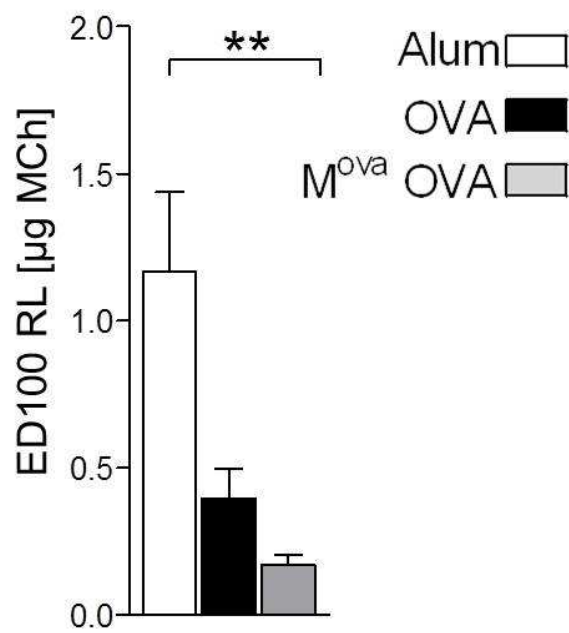
Fig. S2

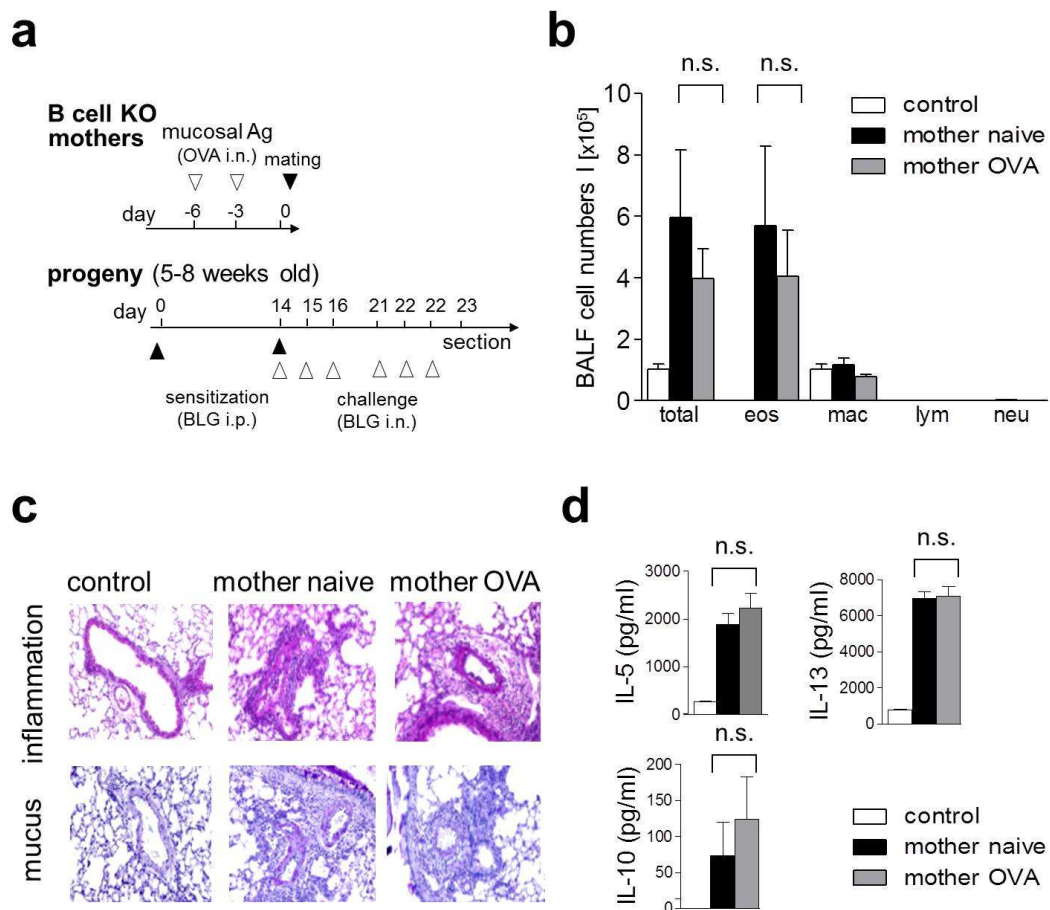
Fig. S3

Fig. S4

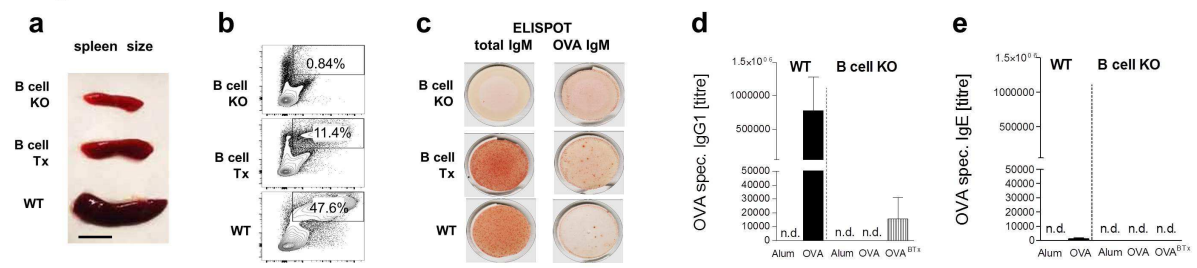


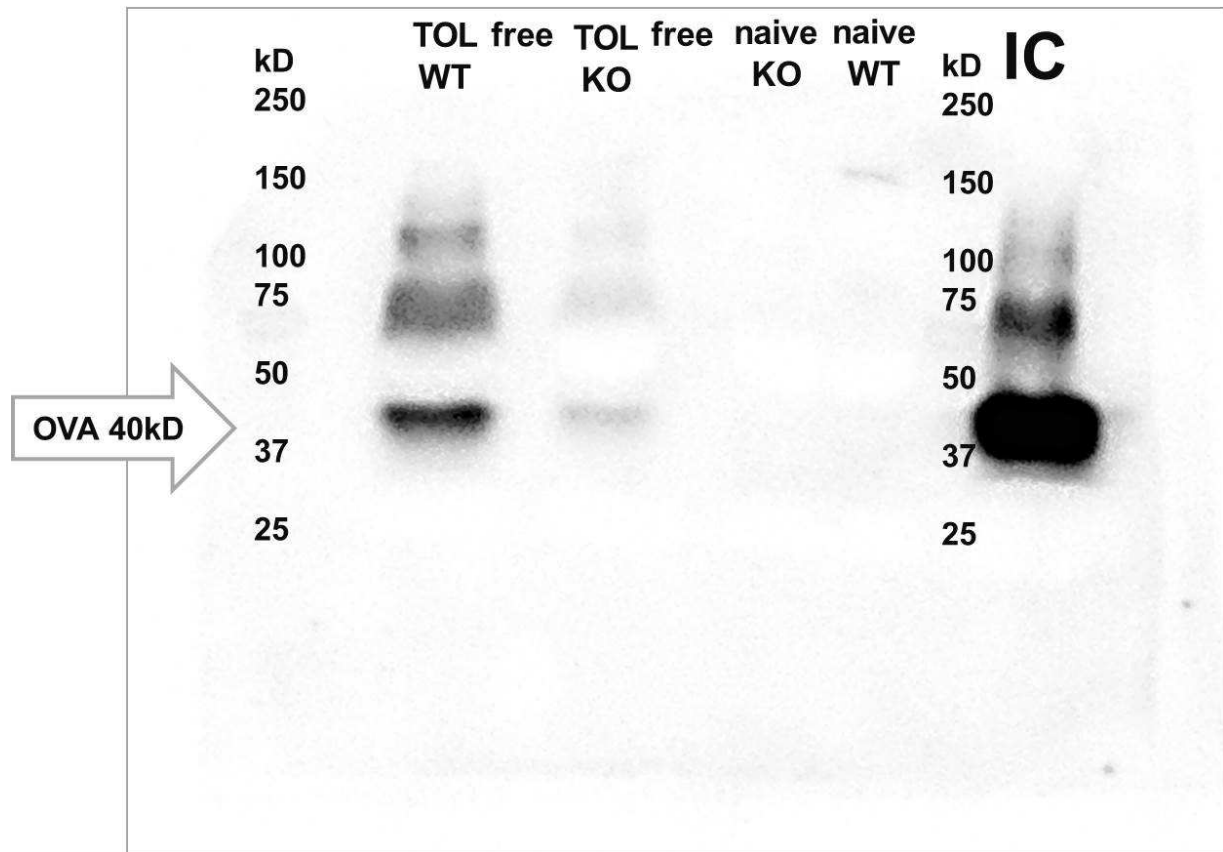
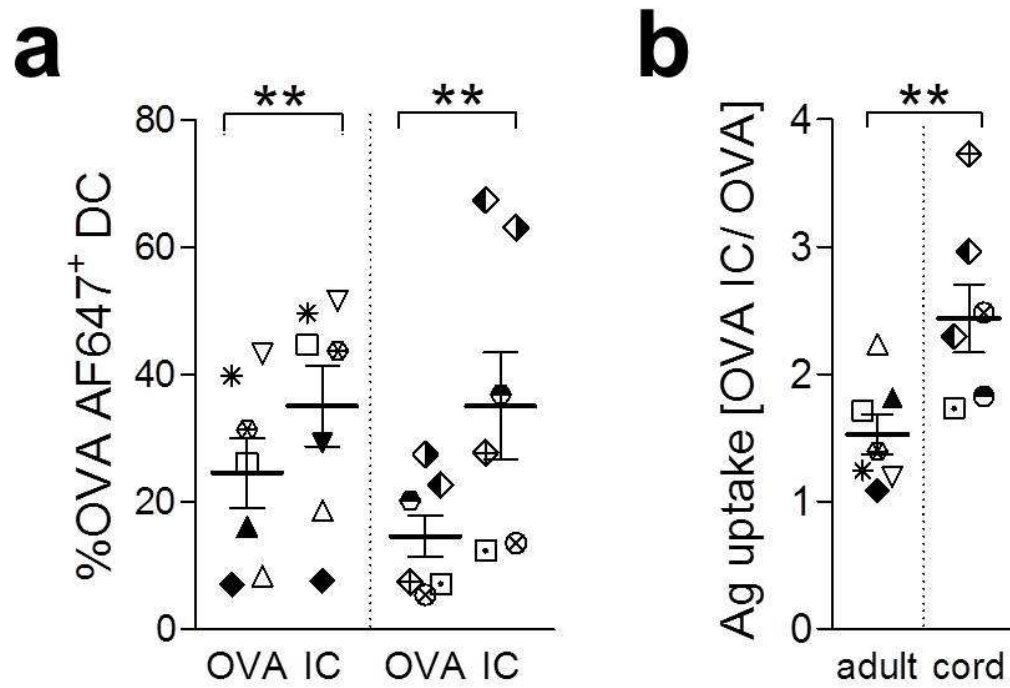
Fig. S5

Fig. S6

Online repository figure legends

Fig. S1: B cell deficient mice lack CD5⁺ regulatory B cells but still can be tolerized in a murine model of allergic airway inflammation

(a) Lacking population of CD19⁺CD5⁺ B cells in the spleens of B cell deficient mice (1 experiment with 19-26 mice/group), (b) experimental scheme, (c) reduced BALF cell counts and BALF eosinophils (eos), macrophages (mac), lymphocytes (lym), neutrophils (neu), (d) reduced lung inflammation and mucus production (PAS/HE staining of paraffin-embedded lung-slices) in tolerized compared to allergic B cell knockout mice, (e) reduced concentration of cytokines in cell culture supernatants after antigen-specific restimulation of bronchial lymph node cells (one representative experiment with 2-6 mice/group, mean + s.e.m., control: control group; allergic: OVA-allergic group tolerized: OVA-tolerized group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Fig. S2: Increased airway hyperreagibility in the offspring of B cell deficient mice that received OVA mucosally before mating

Aggravated airway hyperreagibility in B cell KO mice after maternal OVA administration (ED: effective dose to elicit significant increase of lung resistance (RL) in invasive lung function measurements, one experiment with $n \geq 12$ mice per experimental group, * $P < 0.05$).

Fig. S3: Proallergenic effect of maternal immunization in B cell deficient mice is antigen-specific

(a) Experimental scheme. (b) BALF total cell count, and BALF eosinophils (eos), macrophages (mac), lymphocytes (lym), neutrophils (neu); (c) Lung inflammation and mucus production, as evaluated by HE and PAS staining; (d) Antigen-specific cytokine production. (control: control group; mother naive: BLG-allergic offspring of naïve dams; mother OVA: BLG-allergic offspring of OVA-tolerized dams; mean + s.e.m., $n \geq 4$ mice per experimental group from two experiments, n.s. not significant).

Fig. S4: Successful engraftment of B cells in B cell deficient recipient mice

(a) Increased spleen size and (b) detection of B220⁺IgD⁺ cells in bone marrow flow cytometry of transplanted B cell deficient mice more than two months after transplantation and mating, (c) total and OVA specific IgM production as assessed by ELISPOT assays of splenocytes, (d) OVA specific IgG, but no (e) OVA specific IgE production after B cell transfer (1-3 representative mice from at least two experiments with $n \geq 5$ mice/group).

Fig. S5: Detection of OVA in the amniotic fluid of treated WT and B cell deficient mice

(a) Increased OVA amount in the amniotic fluid of WT mice that received OVA preconceptionally compared to amniotic fluid of equally treated B cell deficient mice (full unedited Western Blot).

Fig. S6: Donor individual data for IC uptake in human adult versus cord blood DCs including information on atopy status

(a) Antigen uptake in human adult versus cord blood DCs after incubation with OVA or OVA IC as shown in main Fig. 5, individual donors can be tracked by respective data point formattings (donor ▲ suffers from mild atopic dermatitis, donor ◆ suffers from allergic rhinitis, all other donors are non-atopic); (h) ratio of OVA IC/ OVA antigen uptake from individual donors with same individual data point markings as in (a), n=7 donors per group, 3 independent experiments. All bars display mean + s.e.m., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.