

IgE knock-in mice suggest a role for high levels of IgE in basophil-mediated active systemic anaphylaxis

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Immunoglobulin E (IgE) production is tightly regulated at the cellular and genetic levels and is believed to be central to allergy development. At least two cellular pathways exist that lead to systemic anaphylaxis reactions *in vivo*: IgE-sensitized mast cells and IgG1-sensitized basophils. Passive anaphylaxis, by application of allergen and allergen-specific antibodies in mice, indicates a differential contribution of immunoglobulin isotypes to anaphylaxis. However, analysis of a dynamic immunization-mediated antibody response in anaphylaxis is difficult. Here, we generated IgE knock-in mice (IgE^{ki}), which express the IgE heavy chain instead of IgG1, in order to analyze the contribution of IgG1 and IgE to active anaphylaxis *in vivo*. IgE^{ki} mice display increased IgE production both *in vitro* and *in vivo*. The sensitization of IgE^{ki} mice by immunization followed by antigen challenge leads to increased anaphylaxis. Homozygous IgE^{ki} mice, which lack IgG1 due to the knock-in strategy, are most susceptible to active systemic anaphylaxis. The depletion of basophils demonstrates their importance in IgE-mediated anaphylaxis. Therefore, we propose that an enhanced, antigen-specific, polyclonal IgE response, as is the case in allergic patients, is probably the most efficient way to sensitize basophils to contribute to systemic anaphylaxis *in vivo*.

Keywords: Active systemic anaphylaxis · Allergy model · Basophils · IgE knock-in



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Introduction

Allergy has become a major threat to public health in developed countries [1, 2]. In particular, systemic anaphylaxis, which

is a rapid and often fatal allergic reaction to a systemic allergen exposure, e.g. a bee sting into the vascular system, necessitates a fundamental understanding of the immune parameters involved.

The causative association of allergen-specific Immunoglobulin E (IgE), the high-affinity IgE receptor (FcεRI), and mast cells for immediate type allergy and anaphylaxis has been studied for

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decades [3–5]. However, since the discovery of anaphylaxis in IgE-deficient mice [6] and more recently studies on basophil biology, a number of publications have focused on the contribution of alternative pathways to anaphylaxis [7–9].

It has become evident that the isotype, quantity, and quality of the sensitizing antibodies are important parameters for anaphylaxis [9]. In summary, at least two mutually nonexclusive pathways exist that employ allergen-mediated cross-linking of either receptor bound IgE and/or receptor bound IgG and lead to activation of mast cells and/or basophils leading to release of inflammatory substances, e.g. histamine or platelet activating factor [7, 10].

Nevertheless, experiments to examine the role of the active polyclonal antibody response in anaphylaxis are hampered by the low expression of IgE and a low frequency of IgE expressing B cells in WT mice [11, 12]. In order to circumvent this problem, we generated an IgE knock-in mouse strain to study the role of IgE regulation in vivo. We created a high-IgE expressing mouse model for allergy research based on work by Rajewsky et al. [13], who showed that the replacement of the murine IgG1 heavy chain locus by human IgG1 leads to humanized antibody production in vivo. We adapted this approach and replaced the exons encoding the soluble part of the constant region of murine IgG1 with the murine IgE counterpart. The advantage of this approach over conventional IgE transgenic mice is twofold. First, it is possible to study the regulatory influences of the genetic region in a defined way, excluding positional effects of the classic transgenic approach. Second, it allows the natural usage of the endogenous variable, diversity, and joining segment of the antibody gene region and, therefore, the generation of polyclonal IgE antibody responses against any given antigen and not only the monoclonal IgE production against a single model antigen [13, 14]. Indeed, both IgE and IgG1 are dependent on Th-2 type T-cell and cytokine signals, e.g. CD40–CD40L interaction and IL-4. However, a number of studies suggest that the developmental switch to IgE has unique features as it can occur outside secondary lymphoid structures [15] or initiate in germinal centers (GCs) and rapidly progresses to IgE⁺ plasma cells located outside the GC [16]. Recently, membrane IgE GFP-reporter mouse strains suggested a scenario where IgE⁺ B cells develop with similar kinetics compared with those of IgG1⁺ B cells, but without an IgG1⁺ intermediate stage. It was reported that while the initial phase is GC-dependent, IgE⁺ memory B cells show an atypical propensity to differentiate into short-lived IgE plasma cells, thereby downregulating the IgE response [11, 12]. These cellular differences, but also genetic differences like the IgE-specific 3'-region with the membrane exons and the polyadenylation sites critically determine the low expression of IgE [17]. These IgE-specific features keep the expression of IgE several orders lower than that of IgG, reflecting fundamental differences in biologic function between these two immunoglobulins. IgE binds with very high affinity to FcεRI on basophils and mast cells [18]. It is an integral part of the defense mechanisms against large extracellular parasites, e.g. helminths, and is misdirected in the case of allergy [19, 20]. Conversely, IgG subclasses can activate and inhibit a wide range of cells, including basophils and mast cells, by the engagement of activating and inhibiting Fcγ receptors [18, 21, 22].

Here, we present evidence that the genetic regulatory regions of IgG1 act on the newly positioned IgE gene. We provide data that IgE secretion is particularly upregulated in vivo in antigen-specific IgE responses. While increased passively bound IgE could be detected on basophils and B cells, backcrossing to CD23 (FcεRII, low affinity IgE receptor)-deficient mice [23] abolished the detection of surface IgE⁺ B cells. However, in vitro class switch induction results in increased bona fide membrane IgE expression in cells from the IgE knock-in (IgE^{ki}) mice, which is similar to IgG1 expression in WT mice. This suggests that an undefined mechanism might exist in vivo, which limits the expression of IgE⁺ B cells. Finally, active systemic anaphylaxis is most severe in homozygous IgE knock-in mice. This suggests that in vivo increased IgE, but not IgG1, is an efficient trigger of anaphylaxis. Depletion experiments implicate basophils as an important cell population in the IgE-dominated active systemic anaphylaxis.

Results

Introduction of the soluble part of the IgE heavy chain into the IgG1 locus

The goal of the genetic manipulation of the mouse germline was to express the IgE immunoglobulin devoid of its tight genetic control [24]. Depending on the mouse strain, IgG1 is expressed in serum up to 200 times higher than IgE. Furthermore, after Th-2 polarization, B cells express high amounts of IgG1 on the membrane, whereas membrane IgE-expressing B cells are rarely seen. Therefore, we reasoned that, by replacing the IgG1 heavy chain exons by IgE, we could transfer the regulatory mechanism of IgG1 to IgE. In the targeting construct, the exons encoding the soluble form of IgE are preceded by the IgG1 class switch region and downstream by the membrane exons of IgG1 (Fig. 1A). This allows the bona fide regulation of the IgE knock-in in an IgG1-analogous manner. The usage of the membrane exons of IgG1 and its downstream polyadenylation signals was deliberately chosen to release IgE of these important regulatory regions [25]. Embryonic stem cells containing the correct integration were identified by PCR (Fig. 1B) and southern blot (Fig. 1C). The successful germ-line deletion of the loxP-framed IgG1 exons was tested by southern blot (data not shown) and PCR (Fig. 1D). The IgE knock-in mice were then backcrossed to C57BL/6 mice in order to obtain heterozygous (IgE^{wt/ki}) and homozygous (IgE^{ki/ki}) mice.

Increased IgE expression in IgE knock-in mice

Two assays were used to determine the functionality of the genetic manipulation. First, we determined the serum immunoglobulin levels in unchallenged IgE knock-in mice. We compared IgM, IgG1, IgG2b, and IgE from heterozygous and homozygous mice and their WT littermates (Fig. 1E). The serum levels of 2 month old heterozygous mice were not changed for IgM, IgG1, or IgG2b. Surprisingly, we found that the deletion of one of the two IgG1 alleles did not lead to a significant reduction of IgG1 of

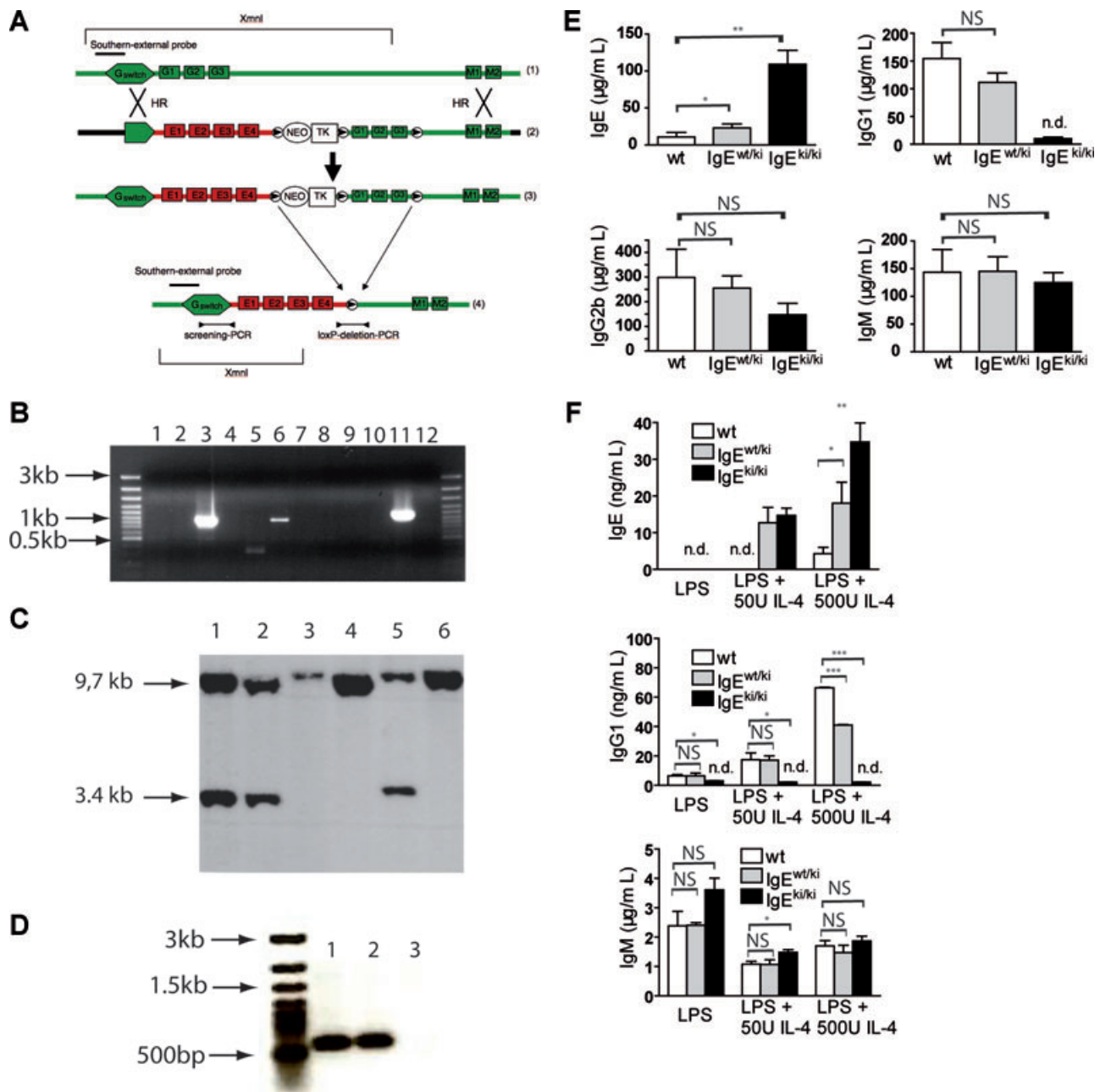


Figure 1. Strategy of the IgE knock-in, screening by PCR, southern blot, and immunoglobulin expression in vitro and in vivo. (A) Schematic strategy to delete the endogenous IgG1 exons (G1–3) from the wild-type genomic configuration (1). The target vector (2) consists of the four IgE exons (E1–4), separated by a loxP deletion/insertion by a neomycin resistance cassette (neo) and a thymidine kinase gene (TK), homologous recombination (HR) (3), screening PCR, southern blot with an external probe and a loxP-deletion-PCR. (B) Two individual positive clones from pools of ES cell clones are shown. Lanes 11 and 12 are positive and negative controls, respectively. (C) A southern blot with the external probe of XmnI digested genomic DNA is shown. This identified the successful deletion/insertion by the generation of a 3.4 kbp fragment from a genomic 9.7 kbp WT allele. 1 = Balb I ES clone 146 (+); 2 = Balb I ES clone 92 (+); 3 = E14.1 WT ES control; 4 = Balb I ES control; 5 = E14.1 clone 639 and 6 = E14.1 clone 391 (negative). (D) loxP-deletion-PCR for deletion of floxed selection cassette and IgG1 locus is shown. The left primer is IgE- and right IgG1 specific (see 1A). Homozygous IgE-knock-in (lane 1), heterozygous IgE-knock-in (lane 2), and WT genomic tail DNA (lane 3). (B–D) Data shown are representative of two experiments performed. (E) Serum immunoglobulin levels of different isotypes of matched heterozygous ($n = 12$), homozygous ($n = 5$) IgE knock-in mice and WT littermates ($n = 6$) were determined by ELISA. (F) In vitro stimulation of 1×10^6 spleen cells/mL with LPS (20 $\mu\text{g/mL}$) and LPS + 50 Units or 500 Units IL-4 for 4 days. Measurement of total isotype-specific immunoglobulins by ELISA. Cells from approximately 2-month old WT littermates, heterozygous, and homozygous IgE knock-in mice (representative for $n = 3$ per group, two independent repeats) were tested. Data are shown as mean + SEM of the indicated number of mice and are representative of two experiments performed. n.d. = not detectable, NS = not significant. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, Students t-test.

heterozygous IgE knock-in mice. Only IgE was moderately increased in heterozygous IgE knock-in mice to twofold the normal IgE concentrations. The homozygous IgE knock-in mice displayed a complete absence of IgG1, but a tenfold increase of total serum IgE (Fig. 1E).

Second, we stimulated spleen cells with LPS with or without exogenous IL-4. We used a low dose (50 Units/mL) and high dose (500 Units/mL) regimen, which favors either induction of class switch to IgG1 or IgE, respectively. B cells from WT and IgE^{wt/ki} mice produced comparable levels of IgM and IgG1 in vitro. As predicted, homozygous IgE knock-in spleen cells could not produce IgG1, but produced normal IgM levels in vitro. In line with the genetic manipulation, the IgE production was fundamentally changed in vitro. First of all, WT, IgE^{wt/ki} and IgE^{ki/ki} B cells do not produce IgE when stimulated with LPS alone. However, IgG1 is indeed clearly less dependent on IL-4 as a class switch factor and is produced in low amounts in response to LPS alone and in increased amounts with low dose IL-4 (IgG1 20 ng/mL) (Fig. 1F). In contrast, IgE^{wt/ki} and IgE^{ki/ki} B cells secrete no IgE upon LPS stimulation, but significantly increased concentrations when low dose IL-4 is added (about 12 ng/mL) (Fig. 1F), while WT B cells did not secrete IgE under low dose IL-4. This qualitative change in IgE synthesis is in accordance with the IgG1 levels produced. The quantitative effect is also evident when a high dose IL-4 with LPS is applied. We detected a fourfold higher IgE concentration in the supernatants of spleen cells from IgE^{wt/ki} mice. Spleen cells from IgE^{ki/ki} mice produced sevenfold more IgE than WT cells. In summary the in vivo and in vitro results clearly show that the IgE knock-in is functional. High levels of IgE are synthesized in vitro, which are in the same range as IgG1 (12 ng/mL IgE versus 20 ng/mL IgG1). The in vivo serum IgE levels, on the other hand, are increased, but do not reach the levels of IgG1, presumably due to the reduced in vivo half-life of IgE compared to IgG1 [26].

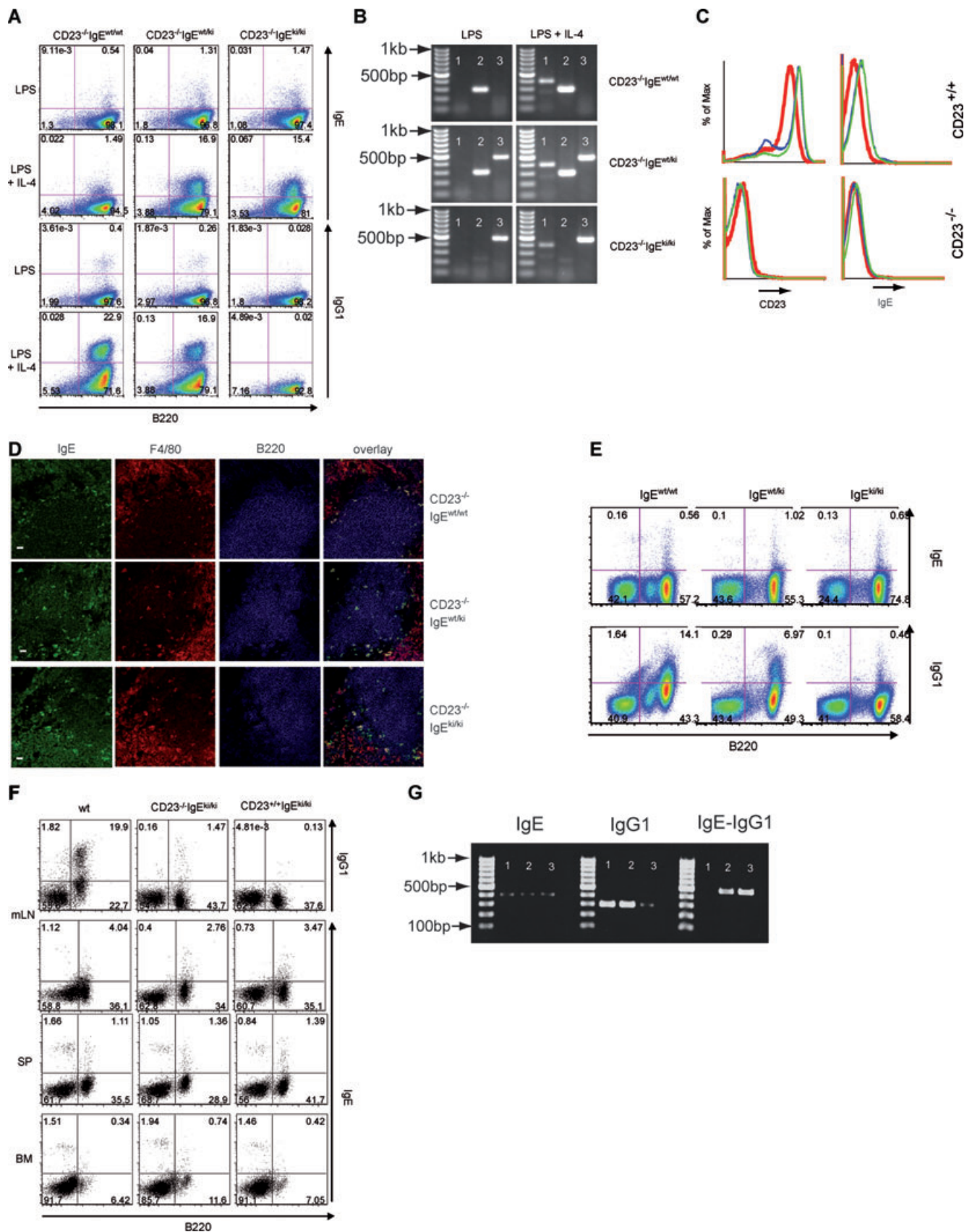
Detection of surface IgE on B cells of IgE knock-in mice in vitro, but not in vivo

The existence of surface IgE positive (memory) B cells in WT mice has only been demonstrated indirectly [27] and has only recently been analyzed by IgE-GFP tagged mice [11, 12, 28]. Even IgE transgenic mice do not display detectable numbers of surface IgE expressing cells in vivo [13]. With the next set of experiments we addressed the question whether surface IgE-positive B cells can be detected in IgE knock-in mice. First, we stimulated total spleen cells for 5 days with LPS and IL-4. We used IgE knock-in mice on the CD23^{-/-} background in order to avoid passive binding of soluble IgE to the low affinity IgE receptor (CD23) on B cells [23]. Surface IgE and IgG1 were detected by flow cytometry. LPS alone neither induced significant IgE nor IgG1 expression (0.4–1.5%) (Fig. 2A and Supporting Information Fig. 1). In B cells from WT mice LPS+IL-4 induces IgG1 (23%), but only very little IgE (1.5%). In contrast, both cells isolated from either heterozygous or homozygous IgE knock-in mice express comparably high amounts

of IgE (ca. 15%) on the cell surface. However, the small fraction of positively stained cells might be due to a cross-reactivity or background staining of the detection antibodies (see also Fig. 2E). WT mice express 23% and heterozygous IgE knock-in mice 10% IgG1 and, as predicted, no IgG1 was found in IgE^{ki/ki} mice. These results suggest that in vitro the chimeric membrane IgE molecule can be transported to the surface with a slightly lower efficiency than natural IgG1. To confirm these results, we performed a RT-PCR analysis of the membrane forms of IgE, IgG1, and the chimeric membrane IgG1-IgE form (Fig. 2B). The results of LPS+IL-4 stimulated cultures are in line with the protein expression data (Fig. 2A); however, LPS alone induces mRNA transcripts with little IgG1 or chimeric IgE being expressed on the surface of the cells (Fig. 2B).

Second, we analyzed B cells from bone marrow, lymph nodes (data not shown), and spleens of heterozygous IgE knock-in mice and their WT littermates. We could find a normal B-cell subset distribution in vivo (data not shown). However, we could not detect membrane IgE-positive B cells (Fig. 2C) in the spleen. The absence of CD23 demonstrates that the increase in IgE expression is not a result of an increase in membrane IgE expressing B cells in unchallenged, naïve mice (Fig. 2C) [23]. Additionally, immunization and boost with the T-dependent antigen trinitro-phenyl-chicken ovalbumin (TNP-OVA) and the subsequent immunohistochemical analysis of splenic B-cell follicles shows only very rare IgE-positive cells located at the edge of the B-cell follicle in IgE knock-in mice of the CD23^{-/-} background (Fig. 2D). Surface IgE and IgG1 expression in vivo were then analyzed after infection with the helminth *Nippostrongylus brasiliensis* (Nb), which leads to pronounced Th-2 responses [29]. Mesenteric lymph nodes of IgE^{ki/ki}, IgE^{ki/wt}, and WT mice were taken at day 14 after infection, at the peak of the germinal center response. IgE^{ki/ki} mice, as expected, showed no staining for IgG1, whereas IgE^{ki/wt} had intermediate expression of surface IgG1 when compared to WT. Unexpectedly, however, membrane IgE expression was not increased in the IgE^{ki/ki} and IgE^{wt/ki} mice (Fig. 2E and Supporting Information Fig. 1). To ensure that acid-stripping of passively bound IgE is not affecting the detection of IgE, we repeated the Nb infection with CD23^{-/-} IgE^{ki/ki}. Again, WT mice express IgG1 (19.9%), whereas only little chimeric IgE can be detected in the mesenteric lymph nodes of CD23^{-/-} IgE^{ki/ki} mice (2.76%). Similar low level IgE expression was seen in spleen and bone marrow of IgE^{ki/ki} mice. PCR reveals the presence of membrane IgE-IgG1 transcripts in spleen (Fig. 2G) without IgE⁺ cells (Fig. 2F).

These findings suggest that IgE-membrane expressing B cells in mice are either rare or cannot be expressed in vivo, even in the IgE-IgG1 chimeric form. In the case of Nb infection, this is fundamentally different from IgG1⁺ B cells, which can readily be detected in lymph nodes of infested mice. In this context, Achatz-Straussberger et al. [31] demonstrated that IgE-secreting B cells, from a different IgE-IgG1 chimeric knock-in mouse called KN1, do migrate more efficiently toward the chemokine CXCL12 into plasma cell niches in the bone marrow. However, in our model bone marrow is not populated more efficiently by IgE⁺ B cells after Nb infestation.



Increased antigen-specific IgE production in immunized IgE knock-in mice

The knock-in strategy allows the natural recombination and selection process for the generation of an antigen-specific polyclonal immunoglobulin response. Immunization with the model antigen TNP-OVA, adsorbed to the Th-2 response favoring adjuvant alum, allowed the comparison of IgE and IgG1 production (Fig. 3A). IgE^{wt/wt} mice produced very little TNP-OVA-specific IgE, but high titers of TNP-OVA-specific total IgG, including IgG1 (Fig. 3B). In contrast, we observed a strong increase of antigen-specific IgE titers in the IgE^{ki} mice compared with that of WT littermates, an 11-fold increase for IgE^{wt/ki} and a 42-fold increase for IgE^{ki/ki}, respectively. IgG1 was not significantly reduced in IgE^{wt/ki}, but is absent in IgE^{ki/ki} mice (Fig. 3B). The genetic deficiency of IgG1 may account for the reduction of total IgG in IgE^{ki/ki} mice, despite the relative increase in IgG2a, IgG2b, and IgG3 levels (Fig. 3B). One further difference between WT and IgE^{ki} is a significant increase in antigen-specific IgM (data not shown). One possible explanation is that CD23-mediated uptake of antigen and a novel mechanism of an antigen transporting capacity by B cells to dendritic cells enhance the specific antigen response, which leads to a stronger immunoglobulin response [30]. This hypothesis is supported by the observation that the changes in CD23^{-/-} IgE knock-in mice for IgG3 and IgG2b are less distinct or absent, respectively (Fig. 3C). Taken together, the IgE knock-in strategy leads to complete lack of IgG1 in homozygous IgE^{ki} mice, reduction of IgG and a very strong specific IgE response in both IgE knock-in genotypes.

Increased active systemic anaphylaxis in IgE knock-in mice

It was recently suggested that basophils have a crucial role in IgG1-mediated anaphylaxis [9]. Therefore, we analyzed naïve mice and observed that in peripheral blood of IgE^{wt/ki} and IgE^{ki/ki} mice twofold more IgE is bound to basophils, while the number of

cells did not increase (Fig. 4A). We conclude that a strong passive saturating binding of IgE to basophils occurs in IgE knock-in mice *in vivo*.

The central experiment to demonstrate a function of increased IgE in allergy is the analysis of anaphylaxis. The three genotypes (Fig. 3A) allowed a dissection of IgE versus IgG1 sensitizing capacity in an active anaphylaxis experiment. We used the same protocol for immunizing IgE knock-in mice as explained above (Fig. 3B), followed by an *i.v.* challenge with 30 µg TNP-OVA to induce systemic anaphylaxis (Fig. 4B). PBS-injected control mice did react with minimal body temperature drop of 0.5°C (Fig. 4B, panel 2). In sensitized IgE^{wt/ki} and IgE^{ki/ki} mice, a comparable strong drop in body temperature of 6°C was observed, whereas WT mice reacted with moderate temperature drop of 4°C. It is important to note that the drop in body temperature in the IgE knock-in mice is more sustained compared with that in WT mice (Fig. 4B, panel 1). Surprisingly, only in the group of the IgE^{ki/ki} mice, about 40%, died due to anaphylaxis (Fig. 4C panel 1). IgE^{ki/ki} lack IgG1 and express high levels of antigen-specific IgE, yet are more susceptible to anaphylactic shock compared with WT mice, which express high levels of antigen-specific IgG1, but little IgE. Therefore, we suggest that antigen-specific IgE is a more potent inducer of anaphylaxis compared with antigen-specific IgG1. Importantly, while the IgE^{ki/ki} and the IgE^{wt/ki} mice had similar temperature curves, death occurred only in the IgE^{ki/ki} mice, arguing for the strongest anaphylactic reaction in the IgE^{ki/ki} mice. These results argue against a major role for the alternative pathway of systemic anaphylaxis, which is mediated largely through IgG1 and FcγRIII and basophil activation in our model [8, 9].

Basophil depletion in double mutant IgE knock-in CD23^{-/-} mice revokes anaphylactic overreactivity

In the following experiment, we addressed two questions, namely, whether CD23, the low affinity receptor for IgE, on B cells in conjunction with the IgE knock-in affects the outcome of systemic

◀ **Figure 2.** *In vitro*, but not *in vivo*, expression of membrane IgE in IgE knock-in mice. (A) Flow cytometry of *in vitro*-stimulated spleen cells from homozygous-, heterozygous IgE knock-in mice, and their IgE WT littermates, all on the CD23^{-/-} background. Four days after LPS, or LPS + IL-4 stimulation cells were stained with anti-IgE or -IgG1. B cells were counterstained using anti-CD45R-B220 (representative for *n* = 2 per group, two independent repeats, see also Supporting Information Fig. 3). (B) *In vitro*-stimulated spleen cells from different genotypes of mice were tested for gene expression of membrane mRNA of WT and chimeric immunoglobulin loci by PCR analysis. Spleen cells stimulated with LPS or with LPS + IL-4 from the three different genotypes (representative for *n* = 2 per group) were tested with IgE (1), IgG1 (2) and chimeric IgE-IgG1 (3) specific primers. (C) IgE and CD23 expression in naïve IgE knock-in mice. CD23 expression (left) and IgE surface expression (right) on splenic B cells (gated on CD45R/B220 positive cells) are shown. IgE^{wt/wt} (red), IgE^{wt/ki} (blue), IgE^{ki/ki} (green). Upper panels are CD23^{+/+} and lower panels are CD23^{-/-} double mutant mice, representative of (*n* = 2 per group, two independent repeats). (D) Immunohistology of spleen of immunized and boosted CD23-deficient IgE knock-in mice. IgE expressing cells (FITC-green), macrophages (F4/80 positive; PE red) and B cells (CD45R-B220; allophycocyanin blue) were acquired with a confocal microscope. Scale bar = 10 µm, and data shown are representative for *n* = 3 per group of a single experiment performed. (E) Flow cytometry analysis of *Nippostrongylus brasiliensis*-infected mice 14 days after infection. Mesenteric lymph node cell suspensions were treated with a mild acid solution to remove soluble IgE bound to CD23. Cells were stained with anti-IgE or anti-IgG1. B cells were counterstained using anti-CD45R. Plots show live cells after doublet exclusion. A representative example of IgE^{wt/wt} (*n* = 3), IgE^{wt/ki} (*n* = 3), IgE^{ki/ki} (*n* = 4) mice is shown (see also Supporting Information Fig. 1) from a single experiment performed. (F) Flow cytometry analysis of 14 days *N. brasiliensis*-infected mice show representative examples. Mesenteric lymph node cells (mLN), spleen cells (SP), and bone marrow cells (BM) were analyzed. Only CD23^{+/+} suspensions, from WT (*n* = 2) and IgE^{ki/ki} (*n* = 2) were treated with a mild acid solution to remove soluble IgE bound to CD23. CD23^{-/-} IgE^{ki/ki} (*n* = 2) cells were not acid treated. Cells were stained with anti-IgE or anti-IgG1. B cells were counterstained using anti-CD45R/B220. Plots show live cells after doublet exclusion. Data shown are from a single experiment performed. (G) PCR analysis of membrane mRNA of WT and chimeric immunoglobulin. Analysis of spleen cells from *N. brasiliensis*-infected mice of different genotypes is shown (representative for *n* = 2, single experiment). Membrane IgE, IgG1, and chimeric IgE-IgG1-specific primers were used. IgE^{wt/wt} (lane 1), IgE^{wt/ki} (lane 2), and IgE^{ki/ki} (lane 3). Lane 3 shows weak background staining for IgG1 transcripts.

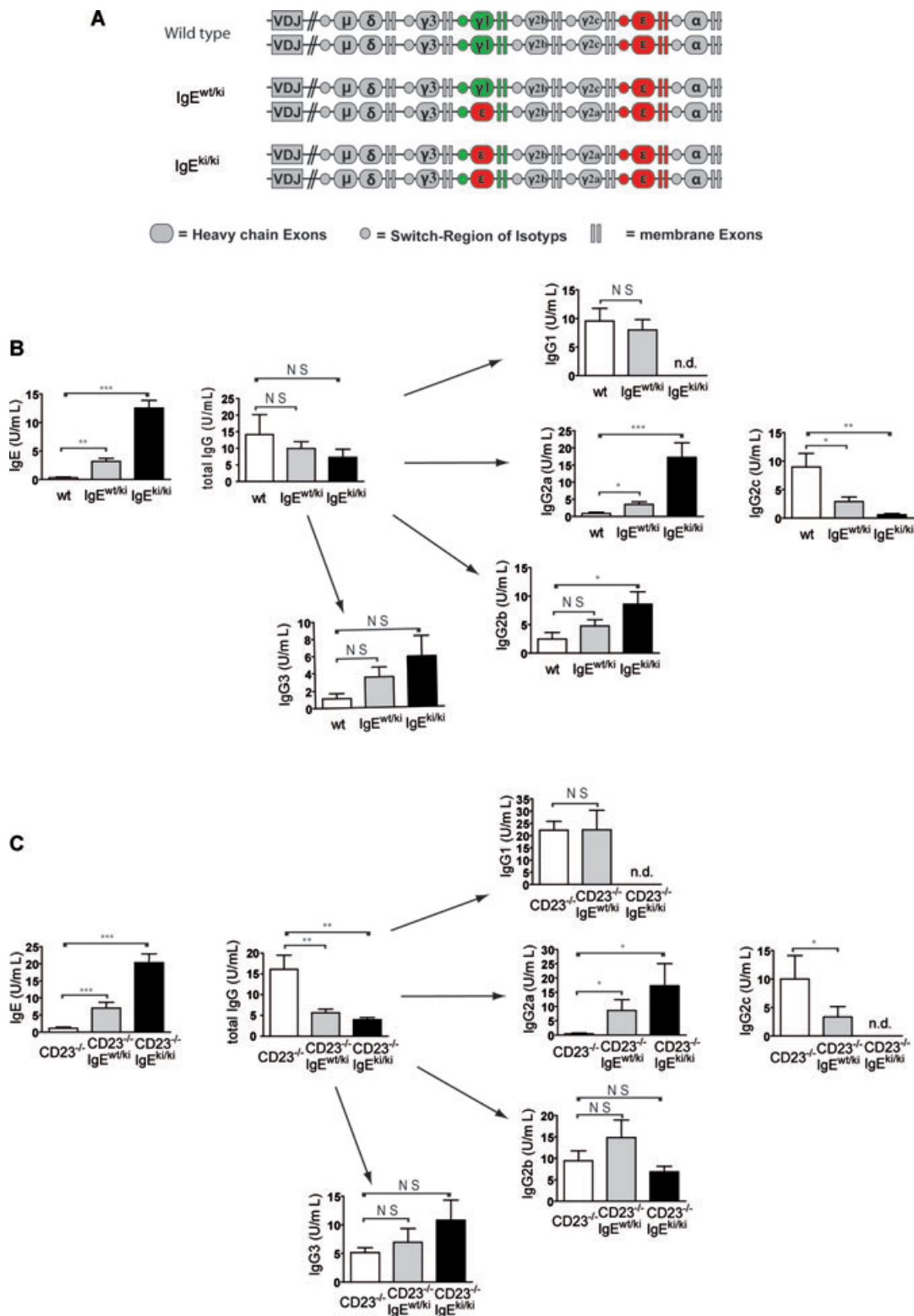
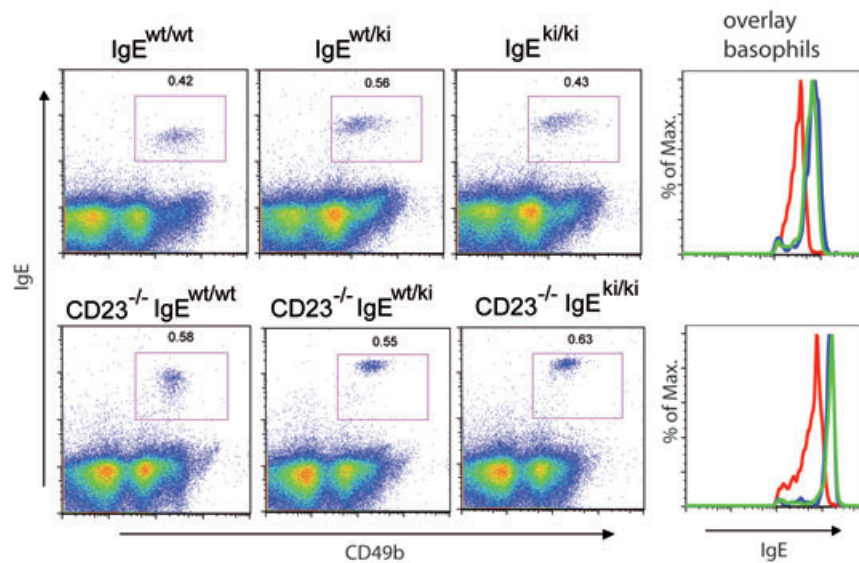
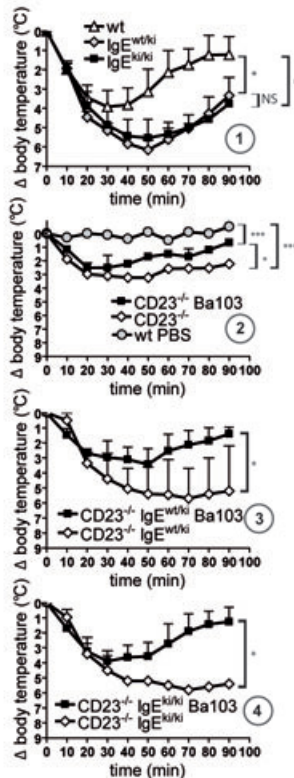


Figure 3. Overview of the immunoglobulin gene locus configuration in IgE knock-in mice and antigen specific immunoglobulin levels in immunized IgE knock-in mice and double mutant CD23-deficient IgE knock-in mice. (A) Immunoglobulin gene locus of the heavy chain in the germline of WT and IgE knock-in mice. (B) TNP-OVA-specific immunoglobulin levels (IgE, total IgG, IgG2a, IgG2c, IgG2b, and IgG3) in the serum of 12-week old WT ($n = 6$), heterozygous ($n = 12$), and homozygous IgE ($n = 5$) knock-in mice or (C) in double mutant CD23-deficient WT ($n = 9$), heterozygous ($n = 5$), and homozygous ($n = 6$) IgE knock-in mice. Data are represented as mean $\pm$ SEM of the indicated number of mice and are pooled from two experiments performed. n.d. = not detectable, NS = not significant. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, Students t-test. Arrows indicate the relationship between total IgG values and IgG subtypes. IgG2a and IgG2c are differentially located on WT and IgE knock-in alleles (see Fig. 3A).

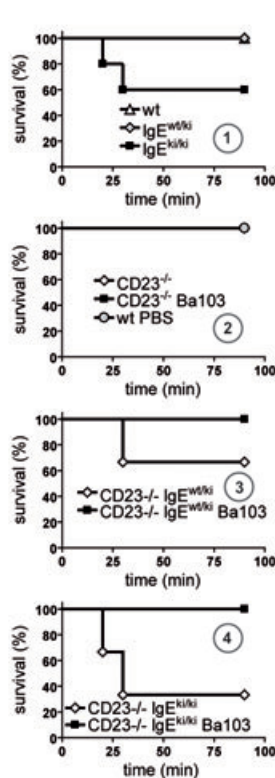
A



B



C



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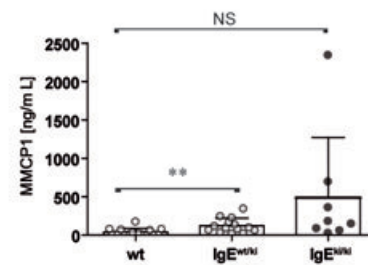


Figure 4. IgE bound to basophils and active systemic anaphylaxis in IgE knock-in mice and double mutant CD23-deficient IgE knock-in mice. (A) Strong IgE staining and CD49b expression on peripheral blood lymphocytes identifies basophils. Upper panels show exemplary staining of single $\text{IgE}^{\text{wt/wt}}$, $\text{IgE}^{\text{wt/ki}}$, $\text{IgE}^{\text{ki/ki}}$ mice ($n = 3$ per genotype). Lower panel shows CD23 $^{-/-}$ IgE knock-in double mutant mice ($n = 3$), of two independent repeats. Histograms show overlay of IgE binding to basophil subpopulations, $\text{IgE}^{\text{wt/wt}}$ (red), $\text{IgE}^{\text{wt/ki}}$ (green), $\text{IgE}^{\text{ki/ki}}$ mice (blue). (B) Anaphylaxis of IgE knock-in mice measured by body temperature (1) and (C) survival curve of WT ($n = 6$), heterozygous ($n = 12$), and homozygous ($n = 5$) mice (1) are shown pooled from two independent repeats, actively immunized and challenged i.v. to induce systemic anaphylaxis. (B) Body temperature and (C) survival curve after the induction of active systemic anaphylaxis of double mutant CD23-deficient WT, heterozygous, and homozygous IgE knock-in mice with and without basophil depletion are shown as mean + SEM of $n = 3$ per group from a single experiment. NS = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (D) Detection of mouse mast cell protease-1 (Mmcp-1) in plasma of anaphylactic mice is shown. Each symbol represents an individual mouse and data shown are pooled from two independent repeats. * $p < 0.0037$; ** $p < 0.0276$, Students t-test.

anaphylaxis, and if basophil depletion influences IgE-mediated active anaphylaxis.

First, we backcrossed the IgE knock-in mice to CD23-deficient mice [23]. No significant effect of a loss of CD23 on anaphylaxis in the IgE^{wt/wt} animals was observed (Fig. 4B panel 2, open squares) when compared with the CD23 competent IgE^{wt/wt} mice (Fig. 4B panel 1, open triangles). Also, no CD23-deficient mice died due to anaphylaxis (Fig. 4C panel 2), similar to wild-type animals (Fig. 4C panel 1). The double-mutant CD23^{-/-} IgE knock-in heterozygous and homozygous mice respond to the anaphylactic challenge with faster and more sustained temperature drop and death (Fig. 4B and C, panels 3 and 4). Again, homozygous CD23^{-/-} IgE^{ki/ki} mice display the strongest increase in lethality. These results implicate that IgE-mediated, active systemic anaphylaxis is moderately increased in CD23-deficient IgE knock-in mice compared with CD23-competent IgE knock-in mice. How CD23 on B cells modulates active systemic anaphylaxis needs further studies. A direct effect of CD23 on effector cells or a proposed negative regulatory function of CD23 on B cells could be involved [23,31].

Because the CD23^{-/-} IgE knock-in mice displayed increased anaphylaxis, we reasoned that they would be better targets to test a potential protective effect of basophil depletion on active anaphylaxis. Therefore, we treated sensitized mice with Ba103 Ab (anti-CD200R3), which depletes basophils, but not mast cells [32] to examine the effect on IgE or IgG1 dominated anaphylaxis. In WT, heterozygous and homozygous IgE^{ki} mice 65, 80, and 85% of basophils (CD49b⁺-IgE⁺) in peripheral blood were depleted, respectively (Supporting Information Fig. 2). The depletion of basophils resulted in reduction of body temperature drop in all three genotypes. This effect was most prominent in IgE knock-in mice in the late phase of anaphylaxis, between 60–90 min. At the endpoint after 90 min the body temperature was 3–4°C lower in untreated mice as compared with that of basophil-depleted mice. In line with this observation, the mortality rate dropped to zero in treated mice. However, at the peak of anaphylaxis around 30–40 min past challenge, basophil-depleted IgE knock-in mice also reacted with substantial anaphylaxis, although they recovered faster than the untreated mice (Fig. 4B and C, panels 3 and 4).

Due to genetic differences between BALB/c mice (where the knock-in was made) and C57BL/6 mice (used for backcrosses) the IgE^{ki/ki} mice express the IgG2a isotype, whereas the WT littermates express IgG2c [33]. This feature of the genetic manipulation is not due to insufficient backcrossing, but results from the close linkage of the IgG isotypes in the immunoglobulin locus. A contribution of differentially expressed antigen-specific IgG2a versus IgG2c (Fig. 3B and C) to the anaphylaxis phenotype, or the moderately increased IgG2b in CD23-competent IgE knock-in (Fig. 3B) is unlikely, because in mice immunized with alum as adjuvant, specific IgG1 is the dominating IgG isotype, resulting in reduced antigen-specific IgG in the IgE knock-in mice [34].

In summary, IgE-sensitized basophils are most likely responsible for the severe body temperature drop in the late phase of anaphylaxis and contribute to death due to anaphylaxis. However, in the early phase of anaphylaxis, sensitized mast cells do have

an important contribution in IgE-dominated systemic anaphylaxis. This is supported by the detection of significantly increased mouse mast cell protease 1 (Mmcp1) in IgE^{wt/ki} mice, but not in IgE^{ki/ki} mice (Fig. 4D). Mmcp1 has been identified as a marker that distinguishes IgE- from IgG-mediated anaphylaxis [7]. As basophils do not express this protease, whereas mast cells do – albeit weakly – [35], this suggests that mast-cell degranulation via IgE may partially contribute to the anaphylaxis phenotype.

Discussion

The IgE knock-in mice are a novel murine model that mimics a deregulation of IgE similar to the enhanced allergen-specific IgE-response in atopic patients [36].

The restriction to the manipulation of the immunoglobulin gene locus allows the dissection of B-cell versus T-cell contribution to the acute allergic phenotype. This new mouse strain allows active immunization experiments to sensitize for anaphylaxis induction. We believe this is closer to the dynamic in vivo situation in allergic patients where polyclonal or oligoclonal antibody responses of different antibody isotypes are induced.

The results presented here suggest that a strong antigen-specific polyclonal IgE response is most powerful in sensitizing both basophils and mast cells. Nevertheless, basophil-depletion experiments indicate that antigen-specific IgE on basophils plays an important role in the anaphylactic process in vivo. This view is indirectly supported by recent data that an IgE-specific hypersensitivity inhibiting molecule called Allergen-1, is expressed on mast cells but not basophils [9]. Mast cells, however, do contribute to the anaphylactic reaction in vivo, since a partial anaphylactic drop in body temperature occurs even in basophil-depleted mice.

Our data are in partial contrast to results, which suggested that basophil-dependent passive systemic anaphylaxis is IgG1 mediated, but not IgE mediated [9,37]. The probable reason for this difference is that passive sensitization with monoclonal IgE is less efficient, due to the instability of IgE, compared with a polyclonal IgE antibody response. Recently, Sawaguchi et al. showed that in a passive systemic anaphylaxis model, mast cell but not basophil depletion inhibited anaphylaxis [38]. In addition, Ohnmacht et al. [40] demonstrated for the Mcpt8Cre-basophil-deficient mouse model that in active systemic anaphylaxis no difference between controls and the basophil-lacking mice exist. This does not contradict our data, because in the IgE^{wt/wt} mice, where IgG1 levels dominate IgE, basophil depletion has only a minimal suppressive effect on anaphylaxis. This supports the hypothesis that basophils are dispensable for an IgG1-dominated anaphylaxis reaction [39]. Studies with novel basophil- or mast cell-deleted mouse strains have to be performed in order to elucidate the precise contribution of basophils versus mast cells in IgE-mediated active anaphylaxis [39,40]. Further support for our model comes from experiments, which suggest that IgG-containing immune complexes inhibit (via FcγRIIB) rather than activate (via FcγRIIA) basophils. They also show an inhibitory effect of IgG on IgE-mediated basophil activation, suggesting that the lack of

an inhibitory signal by IgG1 could contribute to the increased IgE-mediated anaphylaxis we observed in IgE^{ki/ki} mice [18, 21].

First, we used CD23^{-/-} to avoid passive binding of IgE to B cells. However, the result that anaphylaxis is moderately increased in CD23^{-/-} IgE^{ki/ki} mice could imply a regulatory function of CD23 via B cells in anaphylaxis. It is also possible that soluble CD23 forms could directly or indirectly affect the anaphylactic process. It could be interesting to verify our results by analysis of IgE-mediated anaphylaxis in CD23 over expressing transgenic mice [41].

In addition it has been shown that, while CD23 is not expressed on basophils, its expression on B cells might control the size of the free IgE pool [31]. However, our immunization/sensitization experiments suggest that the main difference in specific IgE production results from the IgE knock-in and not from the CD23 deficiency. With regard to anaphylaxis our data suggest that in a low level IgE production in IgE^{wt/wt} CD23^{-/-} mice the depletion of basophils has comparable little influence on anaphylaxis. However, in the strong active immunization induced antigen-specific IgE response, in both IgE^{ki/wt} CD23^{-/-} and IgE^{ki/ki} CD23^{-/-} mice, basophil depletion reduces the anaphylaxis symptoms. Therefore, we postulate that basophils need a complex, polyclonal IgE dominated sensitization to act in systemic anaphylaxis, which is probably not reached in passive IgE sensitization in vivo [38].

The second aspect of the IgE knock-in mice is the lack of IgE⁺ B cells in vivo. The in vitro experiments demonstrate that stimulation of B cells is able to result in high levels of chimeric IgE expression as membrane bound IgE⁺ (mIgE) on B cells. The lack of the IgE⁺ B cells in vivo, in Nb infected mice, implies that either a molecule, which is essential for the expression of mIgE is missing or that an active suppressing factor is inhibiting the expression of membrane IgE⁺ B cells. Whether this observation is merely a genetic artifact or involves unknown IgE regulating mechanism in vivo needs to be addressed in future experiments. Nevertheless targeting of IgE by monoclonal antibodies has become a part of human allergy therapy and might benefit from a better understanding of the in vivo expression or location of membrane IgE-positive cells. Finally, recent data by Yang et al. [11] could partially explain this phenotype by a rapid differentiation of an IgE⁺ B cell into a short-lived plasma B cell.

In summary, we present data on a novel in vivo model allowing a more basic approach to examine genetic effects on the regulation of IgE expression. Its usefulness extends our basic understanding of anaphylaxis by suggesting that IgE sensitization of basophils leads to most severe systemic anaphylaxis reactions. Moreover, this model may become a useful tool in decoding the still enigmatic “beneficial role of IgE” in immune homeostasis [20].

Materials and methods

Generation of the IgE knock-in mouse

We cloned the IgG1 and IgE heavy chain, isolated from 129Sv genomic DNA (Supporting Information Fig. 3) and inserted

between the last exon for soluble IgG1 and the transmembrane exons a loxP site, and after the last exon for soluble IgE a neomycin resistance cassette (Neo^R) and the thymidine kinase (Tk) framed by two loxPs. This target vector was electroporated into the Balb I ES cell line (gift of Birgit Ledermann, Basel, Switzerland). After selection with G418 we performed PCR screening (PCR: 5'-tcaacctacaaacggaagaa and 5'-ctaaacccaacacagaccta). As a PCR control we cloned a similar genomic fragment of the IgG1 region in front of IgE. The test-arm fragment was 155 bp longer, as the actual target vector region, in order to avoid PCR contaminations (Supporting Information Fig. 4). The expected PCR size for controls is 1050 bp and for correct integration of the target vector is 895 bp (Supporting Information Fig. 4). Then, we verified positive clones by southern blots using an external genomic probe (Fig. 1A). Three independent positive clones were injected and chimeric offspring was bred to Deleter mouse strain on the 129Sv genetic background [42]. Testing of the Cre-deletion was done using the primers: 5'-atgggagtttctgtgattc and 5'-gccagaagataagaaaac for the IgE knock-in (PCR-B, 590 bp) (Fig. 1A). After Cre deletion backcrossing for nine generations to C57BL/6 was performed. Some of these mice were then mated to CD23-deficient mice [23] on a C57BL/6 background. All studies with mice were performed in accordance with German animal experimentation law.

ELISA and flow cytometry

Immunoglobulin isotype-specific ELISA was done using goat anti-mouse immunoglobulin anti-sera (Southern Biotech, USA) except for IgE detection, which was done with monoclonal anti-IgE antibodies 84.1C and EM95.3 [43, 44] and total murine IgG, which was done by goat anti-mouse IgG (Jackson, USA). For antigen-specific ELISAs, we coated with 10 µg/mL TNP-OVA. We used pooled sera from immunized mice as a standard and titrated the samples in serial dilutions and gave the titers of specific Igs as relative Units/mL. Anti-CD23 (B3B4, BD Biosciences, USA), anti-CD45RB-B220, anti-IgG1 (Clone A85-1, BD or RMG1-1, Biologend) and anti-IgE (Clone 23G3, BD; or EM95.3) FITC and PE-labeled antibodies were used in FACS analysis on cells that have been preincubated with mouse IgG as Fc block (Jackson Immuno Research, USA) on a FACScalibur (BD Biosciences, USA). For the detection of surface IgE and IgG1 after *N. brasiliensis* infection, mesenteric lymph node cells were prepared by mechanical disruption in 70 µm cell strainers (BD Falcon) and washed with an acid buffer (0.085 M NaCl, 0.005 M KCl, 0.01 M EDTA, and 0.05 M NaAcetate (pH 4)) to remove extrinsic IgE bound to CD23. For the detection of mouse mast cell protease-1 (MMCP-1) in plasma of anaphylactic mice, we used an ELISA kit (eBiosciences, USA) according to the manufacturer.

Antibody production in vitro and in vivo

In vitro antibody production was examined in total spleen cells stimulate with 20 µg/mL LPS, with and without IL-4 (Peprotech, USA) for 4–5 days.

For antigen-specific immune response 3-month old mice were sensitized by injection with 100 µg TNP-OVA (Biosearch Technologies, USA), precipitated with alum (Serva, Heidelberg, Germany), subcutaneously and i.p. After 14 days mice received a similar booster injection. After an additional 5 days mice were bled through the tail vein and serum was prepared for ELISA.

PCR detection of membrane immunoglobulin transcripts

RNA was isolated from in vitro-stimulated splenocytes, cultured for 4 days with LPS with/without IL-4. Total RNA was prepared by Trizol (Sigma, USA) extraction. cDNA was prepared using a kit (Biorad, USA) and PCR was done with primer pairs spanning from the constant region to the transmembrane exons of murine: IgG1 (CAACTGGGAGGCAGGAAATA and GCTTGCCCAATCATGTTCTT), IgE (GGCAAACATGATCTCAAACAGC and TGTTGGCATAGTCTTGAAGG), and the chimeric IgE-IgG1 (GCATAGTGGACCACCCTGAT and GCAGGAAGAGGCTGATGAAG). Bands were visualized on 1.5% agarose gels.

N. brasiliensis infection

Third-stage larvae (L3) of *N. brasiliensis* were recovered from the cultured feces of infected rats, washed extensively in sterile 0.9% saline (37°C), and injected (500 larvae) into mice subcutaneously at the base of the tail.

Mice were provided with antibiotics-containing water (2 g/L neomycin sulfate, 100 mg/L polymyxin B sulfate; Sigma-Aldrich) for the first 7 days after the infection.

Active systemic anaphylaxis and basophil depletion

For anaphylaxis experiments 3-month old mice were sensitized by injection with 100 µg TNP-OVA (Biosearch Technologies, USA), precipitated with alum, subcutaneously and i.p. After 14 days mice received a similar booster injection. After an additional 7 days, mice were injected with 30 µg of antigen i.v. and rectal body temperature was measured every 10 min for 90 min. After the experiment, mice were sacrificed and plasma and organs obtained for further analysis. For statistics, we used the Students *t*-test and GraphPad Prism software. Basophil depletion was done by i.v. injection of 30 µg anti-CD200R (Ba103 mAb, present of H. Karasuyama and Hycult biotech, Germany) 24 h before FACS analysis or anaphylaxis induction.

Acknowledgements: We thank Lisa Wiegand and Hendrikje Drexler for excellent technical assistance, Annika Arendt for prac-

tical assistance, the late Gernot Achatz, Markus Schnare for helpful discussion and Braxton Norwod for help with the manuscript. We particularly acknowledge the most helpful advice by Friederike Jönsson, Paris. We thank Hajime Karasuyama, Tokyo, Japan for generous gift of purified Ba103 mAb. This work was supported by a DFG grant Yu 47/1-1 to P.Y. and ERC grant (PAS.241506) to D.V. and A.T.-N.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

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- Abbreviations:** GC: germinal center · IgE^{Ki}: IgE knock-in · Nb: *Nippostrongylus brasiliensis* · TNP-OVA: trinitro-phenyl-chicken ovalbumin
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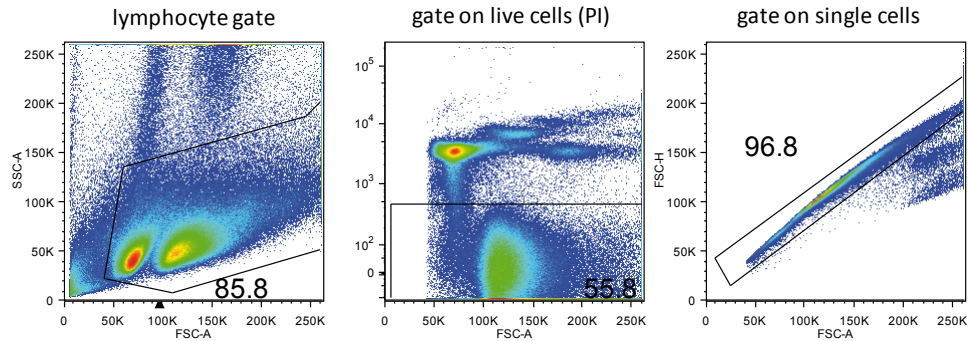
Received: 11/5/2012

Revised: 16/1/2013

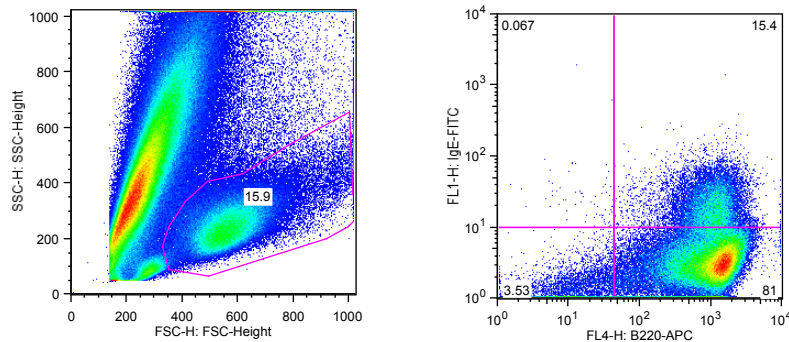
Accepted: 14/2/2013

Accepted article online: 19/2/2013

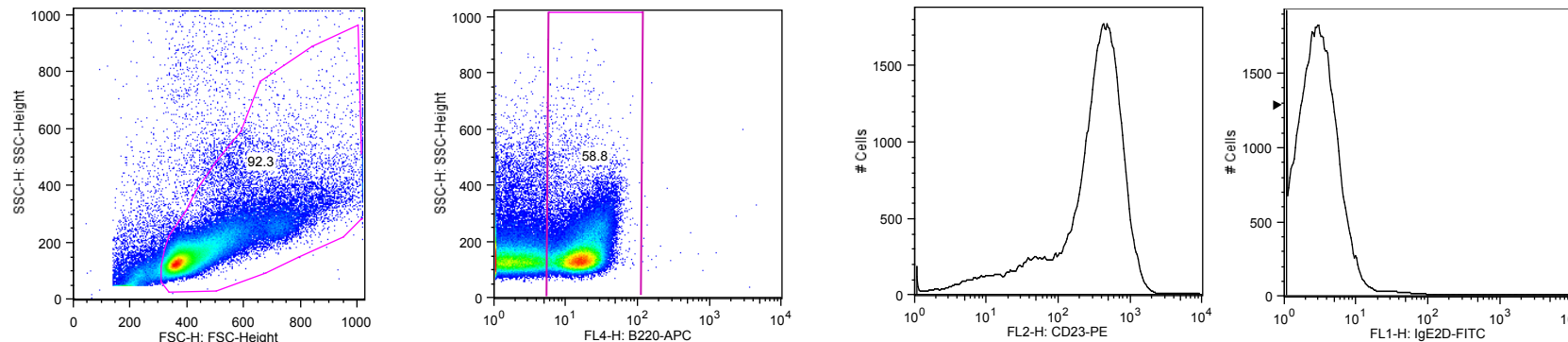
Nippostrongylus brasiliensis (Fig.2E)



In vitro LPS+IL-4 stimulated spleen cells (Fig. 2A)

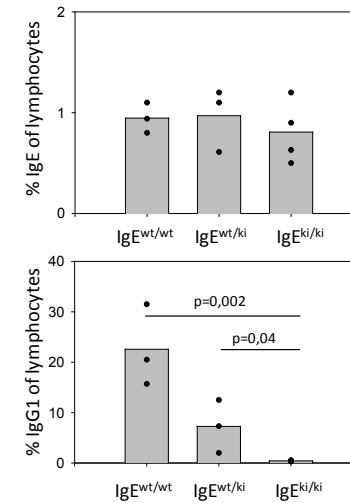


CD23 and IgE expression (Fig. 2C)

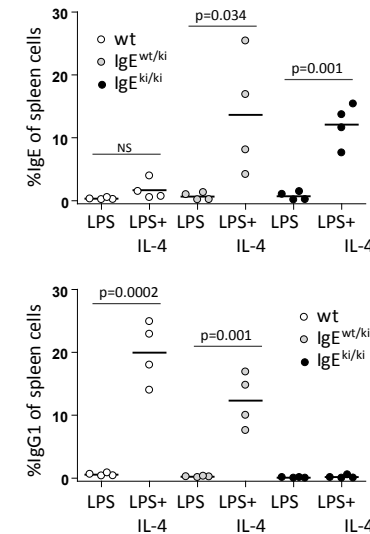


Supporting Information Fig. 1

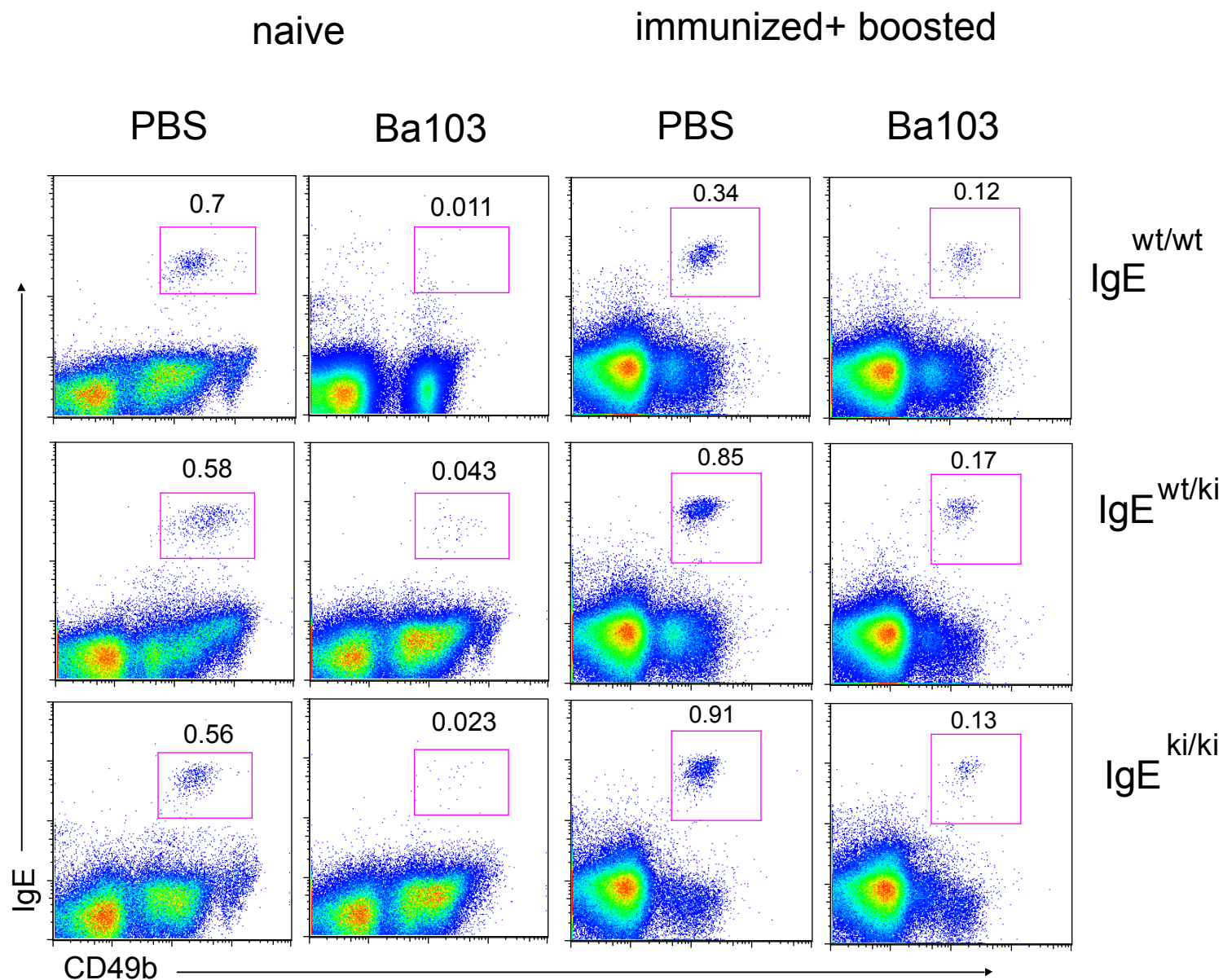
Gating strategies for FACS analysis and summary of surface IgG1+ and IgE+ stainings. Upper panels gating strategy for Nb infection. Right upper graph shows % IgG1+ cells of total lymphocytes, the lower right graph shows % IgE+ cells of total lymphocytes, statistical analysis was done using the student's t test.



Middle panels show in vitro stimulated spleen cells (LPS or LPS+IL-4). Upper, middle right panel shows percent IgE + cells and lower panel shows percent IgG1 positive cells (see also Fig. 2A)



Lower panels show gating strategy for CD23 and IgE expression of CD45RB/B220 positive spleen cells (Fig. 2C).



Supporting Information Fig. 2

Depletion of basophils in wild type and IgE knock in mice. Exemplary FACS of peripheral blood cells of naïve mice treated with 30µg Ba103 (in 100µl PBS) or PBS alone i.p. injection 24h before. Over 90% of basophils (CD49b⁺, IgE⁺) are depleted [9]. Right panels TNP-OVA immunized and boosted mice were injected with 30µg Ba103 (in 100µl PBS) or PBS alone 24h before FACS analysis. In wild type, heterozygous and homozygous IgE^{ki} mice 65%, 80% and 85% of basophils (CD49b⁺-IgE⁺) in peripheral blood were depleted, respectively. n=2, single experiments.

Supporting Information Fig. 3

Sequence comparison between IgE knock in targeting vector (Construct), published Balb/c and C57BL/6 sequences of the IgE heavy chain. The difference between Construct and Balb/c are marked yellow and between Balb/c /Construct and C57BL/6 are marked in blue. The analysis suggests that the IgE knock in is of allotype IgEa, derived from a genomic clone of 129Sv. Balb/c is also IgEa.

Construct	TCTATCAGGAACCCCTCAGCTCTACCCCTGAAGCCCTGTAAAGGCACCTGCTTCCATGACC	60
Balb/c	TCTATCAGGAACCCCTCAGCTCTACCCCTGAAGCCCTGTAAAGGCACCTGCTTCCATGACC	60
Bl6	TCTATCAGGAACCCCTCAGCTCTACCCCTGAAGCCCTGTAAAGGCACCTGCTTCCATGACC	60
Construct	CTGGGCTGCCTGGTAAAGGACTACTTCCCTGGTCTGTGACTGTGACCTGGTATTTCAGAC	120
Balb/c	CTGGGCTGCCTGGTAAAGGACTACTTCCCTGGTCTGTGACTGTGACCTGGTATTTCAGAC	120
Bl6	CTGGGCTGCCTGGTAAAGGACTACTTCCCTGGTCTGTGACTGTGACCTGGTATTTCAGAC	120
Construct	TCCCTGAACATGAGCACTGTGAACCTCCCTGCCCTCGTTCTGAACCTCAAGGTCAACACC	180
Balb/c	TCCCTGAACATGAGCACTGTGAACCTCCCTGCCCTCGTTCTGAACCTCAAGGTCAACACC	180
Bl6	TCCCTGAACATGAGCACTGTGAACCTCCCTGCCCTCGTTCTGAACCTCAAGGTCAACACC	180
Construct	AGCCAAGTGACCACTGGGGCAAGTCAGCCAAGAACTTCACATGCCACGTGACACATCCT	240
Balb/c	AGCCAAGTGACCACTGGGGCAAGTCAGCCAAGAACTTCACATGCCACGTGACACATCCT	240
Bl6	AGCCAAGTGACCACTGGGGCAAGTCAGCCAAGAACTTCACATGCCACGTGACACATCCT	240
Construct	CCATCATTCAACGAAAGTAGGACTATCCTAGTTCGACCTGTCAACATCACTGAGCCACC	300
Balb/c	CCATCATTCAACGAAAGTAGGACTATCCTAGTTCGACCTGTCAACATCACTGAGCCACC	300
Bl6	CCATCATTCAACGAAAGTAGGACTATCCTAGTTCGACCTGTCAACATCACTGAGCCACC	300
Construct	TTGGAGCTACTCCATTTCATCCTGGACCCCAATGCTTCCACTCCACCATCCAGCTGTAC	360
Balb/c	TTGGAGCTACTCCATTTCATCCTGGACCCCAATGCTTCCACTCCACCATCCAGCTGTAC	360
Bl6	TTGGAGCTACTCCATTTCATCCTGGACCCCAATGCTTCCACTCCACCATCCAGCTGTAC	360
Construct	TGCTTCATTATTGGCCACATCCTAAATGATGTCTCTGTGAGCTGGCTAATGGACGATCGG	420
Balb/c	TGCTTCATTATTGGCCACATCCTAAATGATGTCTCTGTGAGCTGGCTAATGGACGATCGG	420
Bl6	TGCTTCATTATTGGCCACATCCTAAATGATGTCTCTGTGAGCTGGCTAATGGACGATCGG	420
Construct	GAGATAACTGATACACTTGCACAACTGTTCTAATCAAGGAGGAAGGCAACTAGCCTCT	480
Balb/c	GAGATAACTGATACACTTGCACAACTGTTCTAATCAAGGAGGAAGGCAACTAGCCTCT	480
Bl6	GAGATAACTGATACACTTGCACAACTGTTCTAATCAAGGAGGAAGGCAACTAGCCTCT	480
Construct	ACCTGCAGTAAACTCAACATCACTGAGCAGCAATGGATGTCTGAAAGCACCTTCACCTGC	540
Balb/c	ACCTGCAGTAAACTCAACATCACTGAGCAGCAATGGATGTCTGAAAGCACCTTCACCTGC	540
Bl6	ACCTGCAGTAAACTCAACATCACTGAGCAGCAATGGATGTCTGAAAGCACCTTCACCTGC	540
Construct	AAGGTCACCTCCCAAGGCGTAGACTATTTGGCCCACTCGGAGATGCCAGATCATGAG	600
Balb/c	AAGGTCACCTCCCAAGGCGTAGACTATTTGGCCCACTCGGAGATGCCAGATCATGAG	600
Bl6	AAGGTCACCTCCCAAGGCGTAGACTATTTGGCCCACTCGGAGATGCCAGATCATGAG	600
Construct	CCACGGGGTGTGATTACCTACCTGATCCACCCAGCCCTCGACCTGTATCAAAACGGT	660
Balb/c	CCACGGGGTGTGATTACCTACCTGATCCACCCAGCCCTCGACCTGTATCAAAACGGT	660
Bl6	CCACGGGGTGTGATTACCTACCTGATCCACCCAGCCCTCGACCTGTATCAAAACGGT	660
Construct	GCTCCCAAGCTTACCTGTCTGGTGGTGGACCTGGAAGCGAGAAGATGTCAATGTGAGC	720
Balb/c	GCTCCCAAGCTTACCTGTCTGGTGGTGGACCTGGAAGCGAGAAGATGTCAATGTGAGC	720
Bl6	GCTCCCAAGCTTACCTGTCTGGTGGTGGACCTGGAAGCGAGAAGATGTCAATGTGAGC	720
Construct	TGGAACCAAGAGAAGAACTTCAGTCTCAGCATCCAGTGGTACACTAAGCACCACAT	780
Balb/c	TGGAACCAAGAGAAGAACTTCAGTCTCAGCATCCAGTGGTACACTAAGCACCACAT	780
Bl6	TGGAACCAAGAGAAGAACTTCAGTCTCAGCATCCAGTGGTACACTAAGCACCACAT	780
Construct	AACGCCACAACCTAGTATCACCTCCATCCTGCCTGTAGTTGCCAAGGACTGGATTGAAGGC	840
Balb/c	AACGCCACAACCTAGTATCACCTCCATCCTGCCTGTAGTTGCCAAGGACTGGATTGAAGGC	840
Bl6	AACGCCACAACCTAGTATCACCTCCATCCTGCCTGTAGTTGCCAAGGACTGGATTGAAGGC	840
Construct	TACGGCTATCAGTGCATAGTGGACCCCTGATTTCCCAAGCCCATTTGCGTTCCATC	900
Balb/c	TACGGCTATCAGTGCATAGTGGACCCCTGATTTCCCAAGCCCATTTGCGTTCCATC	900
Bl6	TACGGCTATCAGTGCATAGTGGACCCCTGATTTCCCAAGCCCATTTGCGTTCCATC	900
Construct	ACCAAGACCCAGGCCAGCGCTCAGCCCCGAGGTATATGTGTTCCACCACAGAGGAG	960
Balb/c	ACCAAGACCCAGGCCAGCGCTCAGCCCCGAGGTATATGTGTTCCACCACAGAGGAG	960
Bl6	ACCAAGACCCAGGCCAGCGCTCAGCCCCGAGGTATATGTGTTCCACCACAGAGGAG	960
Construct	GAGAGCGAGGACAAACGCACACTCACCTGTTTGTATCCAGAACTTCTTCCCTGAGGATATC	1020
Balb/c	GAGAGCGAGGACAAACGCACACTCACCTGTTTGTATCCAGAACTTCTTCCCTGAGGATATC	1020
Bl6	GAGAGCGAGGACAAACGCACACTCACCTGTTTGTATCCAGAACTTCTTCCCTGAGGATATC	1020
Construct	TCTGTGCACTGGCTGGGGATGGCAAATGATCTCAACAGCCAGCACAGTACCACAACA	1080
Balb/c	TCTGTGCACTGGCTGGGGATGGCAAATGATCTCAACAGCCAGCACAGTACCACAACA	1080
Bl6	TCTGTGCACTGGCTGGGGATGGCAAATGATCTCAACAGCCATCACAGTACCACAACA	1080
Construct	CCCTGAAATCCAATGGCTCCAATCAAGGCTTCTTCATCTTCAGTCCCTAGAGGTGCGC	1140
Balb/c	CCCTGAAATCCAATGGCTCCAATCAAGGCTTCTTCATCTTCAGTCCCTAGAGGTGCGC	1140
Bl6	CCCTGAAATCCAATGGCTCCAATCAAGGCTTCTTCATCTTCAGTCCCTAGAGGTGCGC	1140
Construct	AAGCACTCTGGACACAGAGAAAACAGTTCACCTGCCAAGTGATCCATGAGGCACCTTCAG	1200
Balb/c	AAGCACTCTGGACACAGAGAAAACAGTTCACCTGCCAAGTGATCCATGAGGCACCTTCAG	1200
Bl6	AAGCACTCTGGACACAGAGAAAACAGTTCACCTGCCAAGTGATCCATGAGGCACCTTCAG	1200
Construct	AAACCCAGGAACTGGAGAAAACATATCCACAGCCTTGGTAACACCTCCCTCCCTCCC	1260
Balb/c	AAACCCAGGAACTGGAGAAAACATATCCACAGCCTTGGTAACACCTCCCTCCCTCCC	1260
Bl6	AAACCCAGGAACTGGAGAAAACATATCCACAGCCTTGGTAACACCTCCCTCCCTCCC	1260
Construct	TCC 1263	
Balb/c	TCC 1263	
Bl6	TCC 1263	

Explanation of size differences between control PCR and Gene Targeting PCR of targeted ES clones

control PCR (Test arm)

Gene Targeting vector (short arm)

[illegible][illegible]

Supporting Information Fig. 4

As a PCR control we cloned a similar genomic fragment of the IgG1 region in front of IgE. The test-arm fragment was 155bp longer, as the actual target vector region, in order to avoid PCR contaminations. The expected PCR size for controls is 1050bp and for correct integration of the target vector is 895bp. Left Sequence depicts the control PCR template (Test arm) and right sequence the PCR part of the Gene Targeting vector (short arm). Cloning vector sequences (red), IgG1 sequences (black), screening PCR primers (cyan), IgE sequences (green), the 5-prime end of the Gene Targeting vector (magenta) and the additional IgG1 sequences in the control PCR construct (blue) are marked.

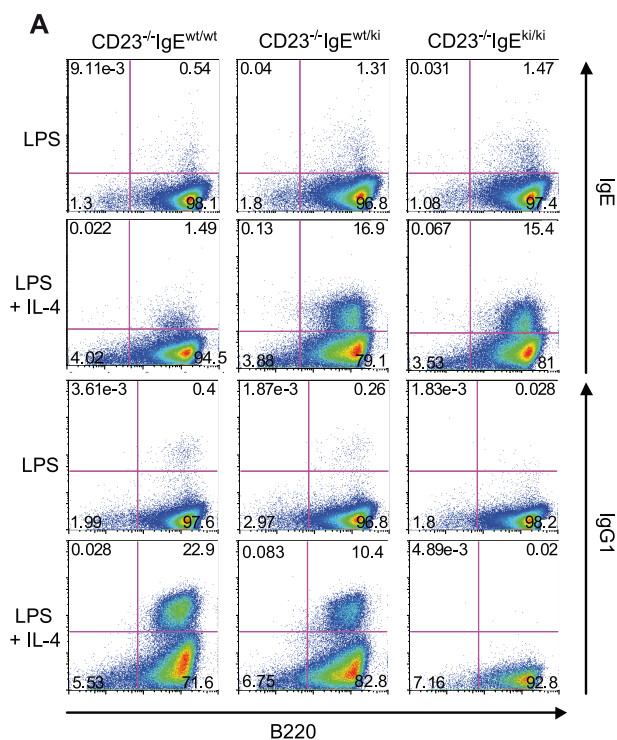
Vol 43 (5) 2013, DOI 10.1002/eji.201242675

IgE knock-in mice suggest a role for high levels of IgE in basophil-mediated active systemic anaphylaxis

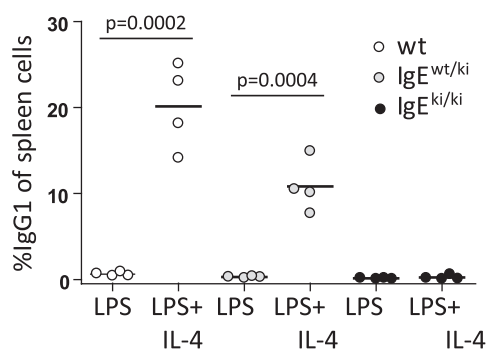
Wolger Lübben, Adriana Turqueti-Neves, Anna Okhrimenko, Christian Stöberl, Volker Schmidt, Klaus Pfeffer, Sonja Dehnert, Sarah Wünsche, Silke Storsberg, Stefanie Paul, Stefan Bauer, Gert Riethmüller, David Voehringer and Philipp Yu

Unfortunately, there are four errors in the above-mentioned article:

1. In the labeling of Figure 2A, middle column ($CD23^{-/-}IgE^{wt/ki}$), bottom flow cytometry panel (LPS+IL-4; B220; IgG1), the % values of the quadrants are identical to those shown in the middle column ($CD23^{-/-}IgE^{wt/ki}$), second plot from top (LPS+IL-4; B220; IgE). The correct Figure 2A is shown here.



2. The mistake in Fig. 2A consequently leads to an incorrect value in the % IgG1 of in vitro stimulated spleen cells (Supporting Information Figure 1). The corrected panel of Supporting Information Figure 1 is shown here and does not change the conclusion of the experiment.



3. The legend of Figure 2A incorrectly refers to “. . . , see also Supporting Information Fig. 3”. The correct statement should be “. . . , see also Supporting Information Fig. 1.”.
4. In the legend to Supporting Information 1, it is noted that the right upper graph shows % IgG1⁺ cells of total lymphocytes, and the lower right graph shows % IgE⁺ cells of total lymphocytes. In fact, the right upper graph shows % IgE⁺ cells and the right lower graph the % IgG1⁺ cells.