

Chapter 3

The Biology of IgE: The Generation of High-Affinity IgE Antibodies

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Abstract B cells can undergo affinity maturation through the process of somatic hypermutation (SHM) followed by selection for high-affinity variants. Affinity maturation occurs in germinal centers (GC) and requires T–B cell cooperation. Extra GC B-cell responses are associated with the production of low-affinity antibodies. B cells producing high-affinity IgE during T-dependent responses are not directly selected in GC but are produced by the sequential switching of GC-selected high-affinity IgG-producing B cells. In contrast, natural low-affinity IgE can be generated without cognate T–B cell interactions in lymphopenic conditions. Low- and high-affinity IgE may differentially affect mast cell survival and degranulation and thus determine whether mast cells contribute to a beneficial or pathogenic environment.

3.1 High-Affinity Versus Low-Affinity IgE Antibodies

B cells can undergo affinity maturation, a process that essentially entails two steps: (1) somatic hypermutation (SHM) of the immunoglobulin genes and (2) selection of the B cells displaying high-affinity variants. Years of evidence have established that affinity maturation occurs in GC. Affinity maturation results in antibodies displaying high-affinity binding for their ligands. However, not all antibody responses undergo affinity maturation, and low-affinity antibodies are also part of some immune responses.

“Natural” IgE antibodies recognize natural antigens such as phosphoryl choline and do not undergo affinity maturation; they are low-affinity IgE antibodies. Importantly, these low-affinity IgE antibodies can be generated without cognate T cell–B cell interactions and without GC, although their generation, like the generation of high-affinity IgE antibodies, is strictly IL-4 dependent [1]. Although the occurrence of natural IgE antibodies have been only demonstrated in conditions of lymphopenia in mice, it is likely that, in a normal individual exposed to antigens

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and allergens, the IgE compartment contains a mixture of natural low-affinity IgE antibodies and cognate T cell–B cell interaction-driven high-affinity IgE antibodies.

As we will discuss in detail later, high-affinity IgE antibodies are generated through a unique developmental pathway that entails the generation of high-affinity IgG1 cells in GC, followed by sequential switching of the IgG1⁺ cells to IgE. Thus, the affinity maturation of the IgE response occurs during the IgG1 phase that precedes sequential switching. Cognate T cell–B cell interactions are essential for the generation of high-affinity IgE antibodies [2].

The functional differences between high- and low-affinity IgE antibodies are highly significant. High-affinity antibodies can be cross-linked by minute amounts of multivalent antigen, whereas low-affinity antibodies require a much higher antigen concentration of such antigens. Thus, antigens can easily cross-link B cells that display high-affinity membrane antibodies. High level of cross-linking of FcεRI receptors results in mast cell degranulation as well as cytokine/chemokine secretion by mast cells, whereas low level of cross-linking leads to cytokine and chemokine secretion by mast cells, but no degranulation [3, 4]. Furthermore, Yamasaki et al. expressed CD8/FcRγ chimeric molecules on mast cells from FcRγ-deficient mice (FcRγ is one of the components of FcεRI, also of CD16 and CD64). Strong cross-linking of the CD8/FcRγ chimeric receptor with anti-CD8 antibodies induced mast cell survival and degranulation, whereas weaker stimulation (in terms of anti-CD8 concentration and valency) resulted in survival but not degranulation [4]. As indicated above, cytokines such as IL-3 are produced by mast cells and are important for their own survival. Thus, both high- and low-affinity IgE antibodies are equally capable of carrying out the beneficial effect of IgE on mast cells. However, because of their differential affinity and specificity, the allergen-mediated cross-linking of IgE antibodies bound to FcεRI molecules can only be carried out by high-affinity IgE antibodies, which are driven by cognate T–B interactions. In targeting IgE response against cancer cells, it is important that this IgE response be of high affinity.

3.2 Switching to IgE and Its Control in B Lymphocytes

Of all secreted immunoglobulins, IgE is found at the lowest concentration in normal individuals, suggesting that the production of IgE is strongly regulated. Atopic diseases are characterized by the production of IgE antibodies to common non-infectious environmental or food proteins. The production of allergen-specific IgE antibodies is highly dependent on cognate T cell–B cell interactions involving the co-stimulatory molecules CD40/CD40L and on the cytokine IL-4 [5, 6]. Atopy is also associated with, and depends on, the presence of allergen-specific Th2 lymphocytes.

Naïve B cells express the constant region of IgM; switching to other constant regions (generating IgGs, IgE or IgA) is brought about by B-cell activation. B-cell stimulation with anti-CD40 + IL-4, soluble CD40L + IL-4, LPS + IL-4, or cognate

interaction with Th2 cells leads to IgG1 and IgE class switching in vitro. However, even under these favorable conditions, the percentage of cells switching to IgE is low compared to the percentage of cells switching to IgG1.

Class switching to IgE is preceded by the production of germline transcripts driven by the IL-4-responsive I ϵ promoter. Replacement of the I ϵ promoter and exon with an enhancer/promoter active in all B cells lead to constitutive expression of the C ϵ germline transcript and IL-4-independent switching to IgE [7], and downregulation of I ϵ transcription caused a reduction in IgE switching [8]. Activation of the I ϵ promoter requires binding of phosphorylated STAT6, NF- κ B, Pax5, C/EBP, PU.1, and E2A transcription factors [5, 6, 9]. Switching ultimately occurs through recombination between S regions, repetitive sequences located 5' of the first exon encoding the constant region (Fig. 3.1, S regions depicted in elliptical shape). Switching, thus, entails DNA recombination and is often abbreviated as CSR (class switch recombination, reviewed in [10–12]).

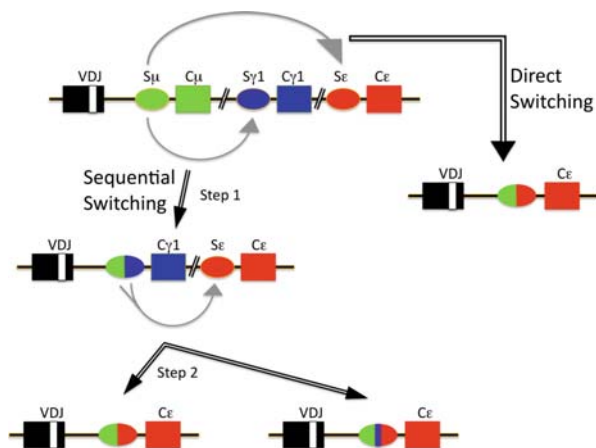


Fig. 3.1 Direct and sequential switching to IgE. Schematic representation of direct (IgM to IgE) and sequential (IgM to IgG1 to IgE) class switching to IgE. The figure depicts in simplified manner relevant elements of the heavy chain immunoglobulin locus: the rearranged VDJ gene, the switch regions (S μ , S γ 1 and S ϵ) and the constant region genes (C μ , C γ 1 and C ϵ)

Germline transcription of IgE and IgG1 in activated B cells can be inhibited by the combination of the cytokines IL-12 and IL-18 [13], IFN- γ [8, 14], TGF- β , bacterial CpG-rich sequences [15], or expression of the proto oncogene Bcl-6 [16]. IL-21 and Id2 also display inhibitory effects on IgE switching. IL-21 will be discussed later in the proposal. Id2 is a transcription factor of the Id family that is involved in the negative regulation of E proteins. Id2-deficient mice have increased IgE levels in serum, and Id2-deficient B cells preferentially switch to IgE in vitro. Id2 is induced by TGF- β in B lymphocytes and suppresses germline I ϵ transcription by interacting with E2A and Pax5 and inhibiting their DNA binding to the I ϵ promoter [9].

A recent study by the Mohrs laboratory showed beyond doubt that, under very strong Th2 stimulation such as the one triggered by helminth parasites, IgE could be induced in μ MT KO mice, which lack mature B cells [17]. However, no IgE was generated in worm-infected J_H -deficient mice. μ MT KO mice have a developmental defect that occasionally can be overcome by expression of other isotypes [18, 19], whereas rearrangement to J_H is physically obligatory for the generation of all immunoglobulins and cannot be overcome. Nevertheless, the result by Mohrs and colleagues indicates that, under strong stimulation, IgE antibodies can be generated without even a membrane IgM⁺ cell phase (please see the section below for more discussion).

3.3 Sequential Switching to IgE in Mice and Humans

In mice and humans, the ϵ locus is downstream the γ loci (Fig. 3.1 is a schematic representation of the mouse locus). In humans there is a duplication of the heavy chain locus that is not found in the mouse. Analysis of the excision circles that occur upon CSR led to the surprising but now indisputable discovery that the switching to IgE in mouse B cells could occur sequentially, that is first a switch from IgM to IgG1, followed by a switch from IgG1 to IgE [20]. Soon after that, Mills et al. found that the switch to ϵ in a human lymphoid cell line was preceded by a $S\mu$ - $S\gamma$ recombination [21]. There have been many subsequent reports in both human and mouse IgE cells [22]. Analysis of the murine chromosomal product $S\mu$ - $S\epsilon$ showed that 30% of the recombined switch regions contained $S\gamma$ 1 sequences, which represented remnants of a previous switch to γ 1 [23]. As shown in Fig. 3.1, this frequency underestimates the real frequency of sequential switching, as many sequentially switched cells do not have an $S\gamma$ remnant (bottom left panel in Fig. 3.1 lacks IgG1 remnants, while bottom center panel in Fig. 3.1 retains a small segment of $S\gamma$ 1 sequence, colored in blue, even though both are generated through sequential switching). In humans, the situation is more complex. Although IgG4 remnants (human IgG4 is considered the equivalent of mouse IgG1) have been found in IgE⁺ cells [24], remnants of other IgGs have also been described [25, 26]. Incontrovertible evidence for direct IgM to IgE switching in humans, collected through the study of excised circles, has been presented [27], but the quantitative comparison of direct versus sequential switching is lacking. Despite the complexity of the situation in humans, the frequency of sequentially switched IgE is roughly the same, with 11 of 40 sequences (27.5%) obtained from patients with schistosomiasis showing IgG remnants [26]. As stated above, both mouse and human studies underestimate the proportion incidence of sequential switching. Importantly, all these molecular data did not address whether sequential switching from IgG1 to IgE occurred immediately or there was a true IgG1 phase on the life of these IgE⁺ cells. Below we review the data indicating that a distinct IgG1 cellular phase can occur in cells that later will become IgE-producing cells.

3.4 Unique Pathway for the Generation of High-Affinity IgE Antibodies

3.4.1 Summary of Findings

Our data show that IgE^+ cells are exceptional because they are largely found outside germinal centers (GC) and express, from very early on, a genetic program of plasma cells (PCs). In contrast, IgG1^+ cells are found in GC and express genes characteristic of the GC stage. In spite of their extra-GC localization and PC phenotype, IgE^+ cells show signs of SHM and affinity maturation. Our data show that high-affinity IgE^+ cells are generated through a unique differentiation program that involves two phases: (1) a pre-IgE-switching phase in which a GC reaction involving SHM and affinity maturation takes place in IgG1^+ cells; (2) after sequential switching of high-affinity IgG1^+ cells to IgE, a post-IgE-switching phase in which IgE cells differentiate swiftly into PCs [28].

In contrast to high-affinity IgE antibodies, low-affinity IgE antibodies are generated outside GC, by direct switching from IgM to IgE. A model of high-affinity IgE versus low-affinity IgE generation is shown in Fig. 3.2. The data that were used to put together the model will be summarized in subsequent paragraphs. Further support for the model in Fig. 3.2 comes from the publication by Zinkernagel's lab in which the generation of natural IgE antibodies (which are low-affinity, poorly mutated antibodies) do not require the presence of organized secondary lymphoid tissue [1].

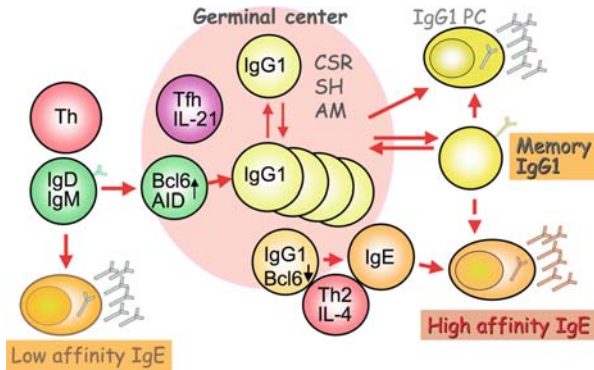


Fig. 3.2 A model of the differentiation of IgE^+ cells in vivo. B lymphocytes in the germinal centers (GC) upregulate Bcl-6, switch to IgG1 and undergo SHM and affinity maturation. Follicular helper T cells (Tfh) provide an IL-21-rich environment in the GC. Bcl-6 expression and signaling through the IL-21R inhibit class switching to IgE in most GC cells. Class switching to IgE can occur in GC cells that downregulate Bcl-6 function, and interact with Th cells in a high IL-4 and low IL-21 microenvironment. Switching to IgE is linked to a pathway of exit from the GC and differentiation to plasma cell. CSR, class switch recombination; SHM, somatic hypermutation; PB, plasmablast; PC, plasma cell; MC, memory cell

3.4.2 Experimental Evidence

The unique pathway involved in the generation of the high-affinity IgE response was uncovered using two mouse models of high IgE response. The first model consists of immunization of T-cell receptor transgenic B-cell knock-in mice, named T-Bmc mice [2]. The second model consists on the infection of wild-type BALB/c mice with the helminth parasite *Nippostrongylus brasiliensis* (Nb) [29, 30]. T-Bmc carry anti-chicken ovalbumin (OVA) T-cell receptor transgenes and anti-influenza hemagglutinin (HA) knocked in B-cell receptor genes on a RAG1-deficient background. The use of T-Bmc enables the tracking of antigen-specific B cells, while the helminth infection of wild-type mice allows the analysis of a broad repertoire response in a non-manipulated immune system.

Even with the availability of these two experimental systems, a technical difficulty remained that hampered the study of the IgE response *in vivo*. Every time there is a high IgE response, flow cytometry analysis using anti-IgE antibodies indicate the vast majority of B cells appear to be IgE⁺ cells, even though molecular analysis show that the vast majority of these cells are not genuine IgE producers. This generalized false-positive result is due to the expression of CD23, the low-affinity ligand for IgE, by B cells. Circulating IgE antibodies, when present at high concentrations, bind via CD23 to genuine IgE⁺ cells as well as IgE⁻ cells, which are most B cells even in the midst of a hyper IgE response. The technical key to overcome the problems in studying IgE⁺ cells was the use of a mild acid treatment that removes the cytophilic IgE antibodies that bind B cells via CD23 [28]. Now the experimental systems and the methodology were in place to reliably study the generation of the high-affinity IgE response *in vivo*.

These technical advantages enabled us to reach the following conclusions regarding the generation of the IgE response. What follows is a summary of the main findings in the manuscript by Erazo et al. [28].

3.4.2.1 IgE⁺ Cells are Found Outside Germinal Centers in Both T/B Monoclonal Mice (T-Bmc) and Wild-Type Mice

This was concluded using a combination of flow cytometry and immunohistology techniques. At no time point after immunization were IgE⁺ cells found in GC. Instead, IgE⁺ cells were found in alternative locations within the spleen and lymph nodes. In contrast with IgE⁺ cells, IgG1⁺ cells were found mainly in GC. Although SHM and affinity maturation occur in GC, our findings do not imply that the IgE response is of low affinity, as we will discuss below.

3.4.2.2 IgE Antibodies Undergo Somatic Hypermutation and Affinity Maturation

The lack of GC localization of IgE⁺ cells, together with their low or absent Bcl-6 and AID expression, raised the question of whether IgE antibodies did undergo SHM and affinity maturation. To study affinity maturation of IgE⁺ cells, we used a variant

peptide that is recognized by the B cells in T-Bmc mice only after affinity maturation of the IgE response. Indeed, upon repeated immunizations we found IgE⁺ cells that could bind to the variant peptide. Furthermore, the sequence of VDJ genes in the variant peptide-binding IgE⁺ cells indicated the presence of three mutations whose presence confers high-affinity binding to the variant peptide.

It was interesting to note that variant peptide-specific antibody titers of both IgG1 and IgE increased over time, with the plateau in IgE titers lagging behind the plateau of IgG1 titers. In addition, IgG1 antibody genes displayed higher frequency of DNA mutations in the VDJ than IgE antibody genes from the same mice. Thus, the high-affinity IgE response lags behind the IgG1 response.

3.4.2.3 IgG1⁺ B Cells can Generate IgE Antibodies by Sequential Switching

Up to this point our results had demonstrated that high-affinity IgE antibodies were produced during a Th2-mediated response *in vivo*, but a GC phase for IgE⁺ cells could not be visualized, generating a potential paradox. Based on these results, and on previous findings of IgG1 switch region remnants on IgE⁺ cells which demonstrated sequential switching from μ to $\gamma 1$ to ϵ [20], we hypothesized that IgE⁺ cells may not undergo SHM and affinity maturation, but rather they could inherit mutated and selected VDJ genes from precursor IgG1⁺ cells. While molecular evidence supporting the existence of sequential switching was abundant, it was equally clear that sequential switching was not obligatory for all IgE responses [31]. Furthermore, it was possible that the second step of sequential switching ($\gamma 1$ to ϵ) occurred immediately after the first step (μ to $\gamma 1$) due to the simultaneous presence of both $\gamma 1$ and ϵ sterile transcripts, without involving an IgG1⁺ cellular phase. It was thus unknown whether cells with a memory IgG1⁺ phenotype could give rise to IgE⁺ cells. To test whether or not memory IgG1⁺ cells could give rise to IgE⁺ cells by sequential switching *in vivo*, we sorted B220⁺Fas⁺IgG1⁺IgD⁻ and B220⁺Fas⁺IgG1⁻IgD⁻ B cells from mice immunized with a variant peptide that does not bind to B cells in T-Bmc mice unless the B cells undergo affinity maturation. Sorted B cells were transferred together with naïve anti-OVA CD4⁺ T cells into T-cell-deficient mice, and the recipient mice were immunized once with the variant peptide. Strikingly, a high-affinity IgE response could be detected only in sera from mice that received the IgG1⁺ B-cell population. In the recipient mice, IgE molecules that, by the experimental design, must be derived from the transferred IgG1⁺ cells carried affinity-enhancing somatic mutations, albeit at lower frequency than IgG1 molecules. These results confirmed that IgG1⁺ cells can generate high-affinity IgE antibodies via sequential switching *in vivo*.

3.4.2.4 Interleukin-21 Inhibits the Sequential Switching of IgG1⁺ Cells to IgE, Thus Inhibiting the High-Affinity IgE Response

While sequential switching to IgE was profoundly suppressed by IL-21, IgG1 production was not. The effect of IL-21 in the low-affinity IgE response was not determined. In GC, IL-21 is produced by follicular helper T cells (Tfh) [32–36].

Although there is disagreement as to some of the many functions of IL-21 (for instance it has been reported that IL-21 induces B-cell death and that it does not induce B-cell death), it has been reported that in vivo injection of IL-21 prevents antigen-specific IgE but not IgG2a production upon immunization [37]. Furthermore, IL-21R-deficient mice have higher production of IgE, but lower IgG1, than wild-type animals [38]. The inhibitory effect of IL-21 on IgE is interesting in that it affects ϵ but not $\gamma 1$ germline transcription. The mechanism by which IL-21 inhibits ϵ germline transcription remains unknown; however, it does not involve inhibition of STAT-6 or NF- κ B activation [37] (Fig. 3.2). IgG1⁺ cells from GC phase were able to produce much higher IgE in response to stimulation in short-time cultures (days 2–5) than purified IgD⁺ cells from naïve or immunized mice. Production of IgE was dependent on stimulation with anti-CD40 antibodies and IL-4 since no IgE was produced in unstimulated cultures, or cultures stimulated with anti-CD40 antibodies and no cytokines. These results can be explained by “de novo” production of IgE by sequential switching of IgG1 cells to IgE in the in vitro cultures. The results are the first demonstration that IL-21 can inhibit the sequential switching of GC IgG1⁺ B lymphocytes to IgE.

3.5 Conclusion

In this manuscript we proposed the hypothesis that high-affinity and low-affinity IgE antibodies are generated through different pathways (Fig. 3.2). High-affinity antibodies can be generated through sequential switching, whereas low-affinity antibodies are generated through direct switching, not involving a GC phase (Fig. 3.2). The existence of different pathways for IgE generation predicts that inhibitors of each of the pathways could be developed to bias the response toward high- or low-affinity IgE. For cancer immunotherapy, the generation of high-affinity antibodies is advantageous; therefore, a selective inhibition of the low-affinity IgE pathway could be beneficial.

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References

1. K. D. McCoy, N. L. Harris, P. Diener, S. Hatak, B. Odermatt, L. Hangartner, B. M. Senn, B. J. Marsland, M. B. Geuking, H. Hengartner, A. J. Macpherson, R. M. Zinkernagel. Natural IgE production in the absence of MHC Class II cognate help. *Immunity* 24: 329–339, 2006.
2. M. A. Curotto de Lafaille, S. Muriglan, M. J. Sunshine, Y. Lei, N. Kutchukhidze, G. C. Furtado, A. K. Wensky, D. Olivares-Villagomez, J. J. Lafaille. Hyper immunoglobulin E response in mice with monoclonal populations of B and T lymphocytes. *J Exp Med* 194: 1349–1359, 2001.
3. C. Gonzalez-Espinosa, S. Odom, A. Olivera, J. P. Hobson, M. E. Martinez, A. Oliveira-Dos-Santos, L. Barra, S. Spiegel, J. M. Penninger, J. Rivera. Preferential signaling and induction of allergy-promoting lymphokines upon weak stimulation of the high affinity IgE receptor on mast cells. *J Exp Med* 197: 1453–1465, 2003.

4. S. Yamasaki, E. Ishikawa, M. Kohno, T. Saito. The quantity and duration of FcRgamma signals determine mast cell degranulation and survival. *Blood* 103: 3093–3101, 2004.
5. H. C. Oettgen, R. S. Geha. IgE regulation and roles in asthma pathogenesis. *J Allergy Clin Immunol* 107: 429–440, 2001.
6. R. S. Geha, H. H. Jabara, S. R. Brodeur. The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol* 3: 721–732, 2003.
7. L. Xu, B. Gorham, S. C. Li, A. Bottaro, F. W. Alt, P. Rothman. Replacement of germ-line epsilon promoter by gene targeting alters control of immunoglobulin heavy chain class switching. *Proc Natl Acad Sci USA* 90: 3705–3709, 1993.
8. L. Xu, P. Rothman. IFN-gamma represses epsilon germline transcription and subsequently down-regulates switch recombination to epsilon. *Int Immunol* 6: 515–521, 1994.
9. M. Sugai, H. Gonda, T. Kusunoki, T. Katakai, Y. Yokota, A. Shimizu. Essential role of Id2 in negative regulation of IgE class switching. *Nat Immunol* 4: 25–30, 2003.
10. J. P. Manis, M. Tian, F. W. Alt. Mechanism and control of class-switch recombination. *Trends Immunol* 23: 31–39, 2002.
11. K. Kinoshita, T. Honjo. Linking class-switch recombination with somatic hypermutation. *Nat Rev Mol Cell Biol* 2: 493–503, 2001.
12. J. A. Fenton, G. Pratt, A. C. Rawstron, G. J. Morgan. Isotype class switching and the pathogenesis of multiple myeloma. *Hematol Oncol* 20: 75–85, 2002.
13. T. Yoshimoto, H. Okamura, Y. I. Tagawa, Y. Iwakura, K. Nakanishi. Interleukin 18 together with interleukin 12 inhibits IgE production by induction of interferon-gamma production from activated B cells. *Proc Natl Acad Sci USA* 94: 3948–3953, 1997.
14. E. Severinson, C. Fernandez, J. Stavnezer. Induction of germ-line immunoglobulin heavy chain transcripts by mitogens and interleukins prior to switch recombination. *Eur J Immunol* 20: 1079–1084, 1990.
15. N. Liu, N. Ohnishi, L. Ni, S. Akira, K. B. Bacon. CpG directly induces T-bet expression and inhibits IgG1 and IgE switching in B cells. *Nat Immunol* 4: 687–693, 2003.
16. M. B. Harris, C. C. Chang, M. T. Berton, N. N. Danial, J. Zhang, D. Kuehner, B. H. Ye, M. Kvatyuk, P. P. Pandolfi, G. Cattoretti, R. Dalla-Favera, P. B. Rothman. Transcriptional repression of Stat6-dependent interleukin-4-induced genes by BCL-6: specific regulation of epsilon transcription and immunoglobulin E switching. *Mol Cell Biol* 19: 7264–7275, 1999.
17. G. Perona-Wright, K. Mohrs, J. Taylor, C. Zaph, D. Artis, E. J. Pearce, M. Mohrs. Cutting edge: Helminth infection induces IgE in the absence of mu- or delta-chain expression. *J Immunol* 181: 6697–6701, 2008.
18. Z. Orinska, A. Osiak, J. Lohler, E. Bulanova, V. Budagian, I. Horak, S. Bulfone-Paus. Novel B cell population producing functional IgG in the absence of membrane IgM expression. *Eur J Immunol* 32: 3472–3480, 2002.
19. M. Hasan, B. Polic, M. Bralic, S. Jonjic, K. Rajewsky. Incomplete block of B cell development and immunoglobulin production in mice carrying the muMT mutation on the BALB/c background. *Eur J Immunol* 32: 3463–3471, 2002.
20. K. Yoshida, M. Matsuoka, S. Usuda, A. Mori, K. Ishizaka, H. Sakano. Immunoglobulin switch circular DNA in the mouse infected with *Nippostrongylus brasiliensis*: evidence for successive class switching from mu to epsilon via gamma 1. *Proc Natl Acad Sci USA* 87: 7829–7833, 1990.
21. F. C. Mills, G. Thyphronitis, F. D. Finkelman, E. E. Max. Ig mu-epsilon isotype switch in IL-4-treated human B lymphoblastoid cells. Evidence for a sequential switch. *J Immunol* 149: 1075–1085, 1992.
22. R. Mandler, F. D. Finkelman, A. D. Levine, C. M. Snapper. IL-4 induction of IgE class switching by lipopolysaccharide-activated murine B cells occurs predominantly through sequential switching. *J Immunol* 150: 407–418, 1993.
23. G. Siebenkotten, C. Esser, M. Wabl, A. Radbruch. The murine IgG1/IgE class switch program. *Eur J Immunol* 22: 1827–1834, 1992.

24. H. H. Jabara, R. Loh, N. Ramesh, D. Vercelli, R. S. Geha. Sequential switching from mu to epsilon via gamma 4 in human B cells stimulated with IL-4 and hydrocortisone. *J Immunol* 151: 4528–4533, 1993.
25. F. C. Mills, M. P. Mitchell, N. Harindranath, E. E. Max. Human Ig S gamma regions and their participation in sequential switching to IgE. *J Immunol* 155: 3021–3036, 1995.
26. B. Baskin, K. B. Islam, B. Evengard, L. Emtestam, C. I. Smith. Direct and sequential switching from mu to epsilon in patients with *Schistosoma mansoni* infection and atopic dermatitis. *Eur J Immunol* 27: 130–135, 1997.
27. K. Zhang, F. C. Mills, A. Saxon. Switch circles from IL-4-directed epsilon class switching from human B lymphocytes. Evidence for direct, sequential, and multiple step sequential switch from mu to epsilon Ig heavy chain gene. *J Immunol* 152: 3427–3435, 1994.
28. A. Erazo, N. Kutchukhidze, M. Leung, A. P. Christ, J. F. Urban, Jr., M. A. Curotto de Lafaille, J. J. Lafaille. Unique maturation program of the IgE response in vivo. *Immunity* 26: 191–203, 2007.
29. I. M. Katona, J. F. Urban, Jr., F. D. Finkelman. The role of L3T4+ and Lyt-2+ T cells in the IgE response and immunity to *Nippostrongylus brasiliensis*. *J Immunol* 140: 3206–3211, 1988.
30. F. D. Finkelman, J. Holmes, I. M. Katona, J. F. Urban, Jr., M. P. Beckmann, L. S. Park, K. A. Schooley, R. L. Coffman, T. R. Mosmann, W. E. Paul. Lymphokine control of in vivo immunoglobulin isotype selection. *Annu Rev Immunol* 8: 303–333, 1990.
31. S. Jung, G. Siebenkotten, A. Radbruch. Frequency of immunoglobulin E class switching is autonomously determined and independent of prior switching to other classes. *J Exp Med* 179: 2023–2026, 1994.
32. C. G. Vinuesa, S. G. Tangye, B. Moser, C. R. Mackay. Follicular B helper T cells in antibody responses and autoimmunity. *Nat Rev Immunol* 5: 853–865, 2005.
33. P. Schaeferli, K. Willmann, A. B. Lang, M. Lipp, P. Loetscher, B. Moser. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med* 192: 1553–1562, 2000.
34. D. Breitfeld, L. Ohl, E. Kremmer, J. Ellwart, F. Sallusto, M. Lipp, R. Forster. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med* 192: 1545–1552, 2000.
35. T. Chtanova, S. G. Tangye, R. Newton, N. Frank, M. R. Hodge, M. S. Rolph, C. R. Mackay. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *J Immunol* 173: 68–78, 2004.
36. A. U. Rasheed, H. P. Rahn, F. Sallusto, M. Lipp, G. Muller. Follicular B helper T cell activity is confined to CXCR5(hi)ICOS(hi) CD4 T cells and is independent of CD57 expression. *Eur J Immunol* 36: 1892–1903, 2006.
37. A. Suto, H. Nakajima, K. Hirose, K. Suzuki, S. Kagami, Y. Seto, A. Hoshimoto, Y. Saito, D. C. Foster, I. Iwamoto. Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. *Blood* 100: 4565–4573, 2002.
38. K. Ozaki, R. Spolski, C. G. Feng, C. F. Qi, J. Cheng, A. Sher, H. C. Morse, 3rd, C. Liu, P. L. Schwartzberg, W. J. Leonard. A critical role for IL-21 in regulating immunoglobulin production. *Science* 298: 1630–1634, 2002.