

What is Unique About the IgE Response?

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Abstract

IgE antibodies are involved in allergic reactions. High affinity IgE antibodies can cause anaphylaxis when cross-linked by minute amounts of antigen. The issue of how the IgE response is initiated and maintained is addressed in this review. A model has been proposed by which IgE⁺ cells expressing antibodies that bind with high affinity to their antigens are generated through an IgG1 intermediate, which goes through affinity maturation in germinal centers (GC) before undergoing sequential switching to IgE. Mice deficient in IgG1 produce IgE at almost normal levels, but the IgE antibodies produced in IgG1-deficient mice lack the antigen-binding strength and the somatic mutations associated with affinity maturation. A GFP reporter strain, which expresses a modified

IgE molecule, was recently developed and was utilized to challenge the sequential switching model. Several molecules that are highly expressed in GC can antagonize class switching to IgE in GC antagonize partially class switching to IgE; in addition, GC IgE⁺ cells are gradually lost from GC as the immune response progresses, as shown with another recently developed, Venus-expressing IgE reporter mouse strain. In contrast, as a population, IgG1 cells thrive in the GC environment. Membrane IgE-expressing plasmablasts and plasma cells (PC) were recognized as a major component of the IgE response in secondary lymphoid organs. The swift development of IgE cells toward the PC fate, together with the affinity maturation of the IgE response via an IgG intermediate, represent the most salient features of the IgE immune responses, which make them distinct from IgG responses.



1. EFFECTOR FUNCTIONS OF IgE ANTIBODIES

Among the five classes (or isotypes) of antibodies (IgM, IgD, IgG, IgE, IgA), the production of IgE is the most tightly controlled. B-lineage cells produce membrane IgE (mIgE) and soluble IgE (sIgE), which has two fates, a very short half-life in the serum compared to other classes of antibodies (Stone, Prussin, & Metcalfe, 2010; Tada, Okumura, Platteau, Beckers, & Bazin, 1975), or binding to cells that express Fcε receptors, thus remaining for weeks on their surface. There are two types of Fcε receptors, the high affinity Fcε Receptor I (FcεRI) and the low affinity FcεRII (CD23). The high and low affinity binding are defined by the interactions of the Fc part of IgE, which is in the Constant region, with the receptors.

1.1. FcεRI

Mast cells and basophils are the major cell types that express the high affinity FcεRI and are prearmed with IgE through binding to the constant (C)-region of IgE. IgE itself regulates the amount of FcεRI molecules present on the cell surface, by stabilizing them at the plasma membrane (Kraft & Kinet, 2007). Cross-linking of IgE molecules by multivalent antigen results in the activation and degranulation of mast cells, releasing histamine and other mediators to induce allergic response (Kinet, 1999; Kraft & Kinet, 2007; MacGlashan, 2008). Cross-linking of FcεRI molecules on basophils also releases cytokines such as IL-4, which has a key role in T_H2 responses (Ben-Sasson, Le Gros, Conrad, Finkelman, & Paul, 1990).

The affinity of IgE molecules for their antigens, determined by the variable regions, is critical. High affinity IgE bound to FcεRI needs only minute

amounts of antigens to be strongly and stably cross-linked, thereby activating the effector mast cells and basophils. In contrast, IgE molecules with low affinity for their antigens can only induce degranulation in the presence of very high levels of antigen, which may not be physiologically achievable. However, these same IgE antibodies with low affinity for antigens are able to increase mast cell survival *in vitro*, probably through weak cross-linking and low levels of intracellular signaling (Asai et al., 2001).

Although the traditional function associated with FcεRI triggering is the result of the release of mediators of allergic inflammation, additional functions have been proposed. IgE-decorated basophils capture soluble antigens and, in a FcεRI-dependent mechanism, release IL-6 and IL-4, which are important for the control of all aspects of the humoral response (Denzel et al., 2008).

Besides mast cells and basophils, other cell types have been reported to express FcεRI. In humans, but not mice, antigen-presenting cells (APC), platelets, and eosinophils express an FcεRI receptor that lacks the β subunit (Kraft & Kinet, 2007). It is possible that the FcεRI expressed by APC can be used to interiorize the IgE-bound antigens that, after processing, are presented to T cells. Human airway smooth muscle cells (ASMC) can express FcεRI, and sIgE induces the release of the proallergy mediator thymic stromal lymphopoietin by ASMC *in vitro* (Redhu et al., 2011). Another cell type that can express FcεRI under special circumstances in the mouse is the neutrophil. FcεRIα knockout mice are resistant to experimental cerebral malaria. Porcherie et al. (2011) showed the recruitment of FcεRI⁺ neutrophils from the bone marrow into the brain. Depletion of neutrophils caused a dramatic improvement in survival of the mice, and, importantly, addition of FcεRI⁺ neutrophils restored cerebral malaria in FcεRIα knockout mice.

1.2. FcεRII (CD23)

The low affinity IgE receptor (CD23, FcεRII) is a protein expressed by all B cells in the mouse. CD23 production is, like IgE itself, also under the control of the IL-4 Signal Transduction and Activator of Transcription (STAT)6 axis, discussed later. CD23 plays an important regulatory role in IgE biology. CD23 expressed on B cells acts as a sink for free IgE in the serum, thus controlling the amount of free IgE (Cheng, Wang, & Locksley, 2010). Consistent with this model, CD23-deficient mice display a twofold increase in serum IgE levels, and a 6- to 12-fold increase in antigen-specific and total

IgE upon immunization with T-dependent antigens (Yu, Kosco-Vilbois, Richards, Kohler, & Lamers, 1994). Less expected were findings related to the immune-stimulatory function of IgE. IgE immune complexes bind CD23^{hi} cells in B cell follicles and enhance antigen presentation and germinal center formation by about one order of magnitude (Hjelm, Karlsson, & Heyman, 2008). The function of CD23 is complex because there are alternative spliced forms, CD23a and CD23b, and CD23 is a membrane protein that can be released from the membrane upon cleavage by ADAM-10 metalloprotease. Each of these forms has its own function in IgE biology (Acharya et al., 2010; Gould & Sutton, 2008).



2. CONTROL OF CLASS SWITCH RECOMBINATION TO IgE

Mature B cells express IgM and IgD on their surfaces. IgE-expressing cells are generated through a DNA recombination process referred to as class switch recombination (CSR). At the molecular level, CSR joins two switch (S) regions, which are separated by up to 170 kb before the CSR event (Fig. 4.1). S regions contain imperfect G-rich repeats and are located upstream of the Constant (C) regions of IgM, all IgGs, IgE, and IgA (Selsing, 2006).

Class switching to IgE is most commonly triggered when T helper 2 (T_H2) or follicular helper (T_{FH}) cells deliver help to B cells. T cell help is mediated by CD40L, which activates CD40 expressed by B cells, and by T_H2/T_{FH}-secreted cytokines, mainly IL-4 but also IL-13, which turn on the ϵ germline transcript (GLT) (sometimes referred to as “sterile”) that originates at the I ϵ promoter (Coffman, Lebman, & Rothman, 1993; Finkelman et al., 1990; Geha, Jabara, & Brodeur, 2003; Rothman et al., 1991; Fig. 4.1). The ϵ GLT splices out the S ϵ region and ends in the C ϵ region (Rothman, Lutzker, Cook, Coffman, & Alt, 1988). IL-4-induced transcription of C ϵ is essential for CSR to IgE (Xu et al., 1993). IL-4-induced signaling results in phosphorylation and dimerization of STAT6, which acts directly on the I ϵ promoter. Another I promoter, I γ 1, also responds to IL-4, and I γ 1 triggering results in switching to IgG1 (Snapper & Paul, 1987), although the IL-4 dependence of switching to IgG1 is less pronounced than that of IgE (Finkelman et al., 1990). Two early IL-4 knockout papers document this phenomenon. Kuhn, Rajewsky, and Muller (1991) reported that IgE antibodies were undetectable in IL-4-deficient mice even upon infection with *Nippostrongylus brasiliensis* (*N.b.*), a strong IgE inducer, whereas nitrophenyl-chicken gammaglobulin (NP-CG)-immunized IL-4 knockout

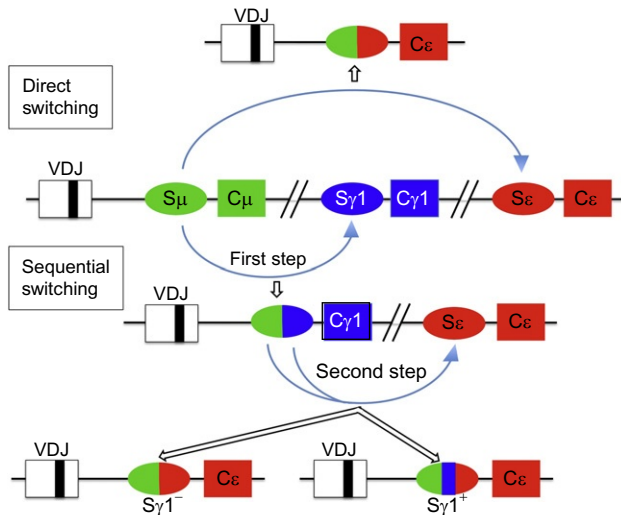


Figure 4.1 Schematic representation of direct and sequential class switch recombination. The preswitched chromosome is depicted in the middle, and the chromosomal products of direct and sequential switching are depicted on the upper and lower parts of the figure, respectively. RNA splicing eliminates I and recombined S regions from the mature immunoglobulin heavy chain mRNA. S γ 1 remnants are always indicative of sequentially switched S μ –S ϵ junctions. [Xiong, Dolpady, Wabl, Curotto de Lafaille, and Lafaille \(2012\)](#).

mice have specific IgG1 antibodies reduced by one-third to one-half. Finally, [Kopf et al. \(1993\)](#) evaluated IgE and IgG1 antibodies upon *N. brasiliensis* infection. They confirmed that IgE antibodies were undetectable, and IgG1 antibodies, while detectable, were reduced 12-fold. Thus, the IL-4 dependence of IgE production is absolute. However, while IL-4 is absolutely essential, T cells are not: as discussed above, basophils and other cells such as eosinophils can produce IL-4.

Although I γ 1 and I ϵ -originated transcripts share a dependency in IL-4/STAT6 signaling, there is much less switching to IgE than to IgG1. This preference cannot be explained by an increased affinity of STAT6 for the I γ 1 promoter. Much to the contrary, phospho-STAT6 binds to the I ϵ promoter with a 10-fold higher affinity than it does to the I γ 1 promoter ([Mao & Stavnezer, 2001](#)). Thus, under limiting IL-4 conditions, switching to IgE should be dominant, which is obviously not the case.

Part of the preference for I γ 1 activation could be explained by binding of E proteins to I ϵ . In addition to the STAT6 binding site, the I ϵ promoter has

two E boxes. Binding of E2A proteins to the E boxes activates I ϵ -initiated transcripts, in cooperative fashion with IL-4. E2A binding to the I ϵ promoter is antagonized by inhibitor of DNA binding 2 (Id2). Accordingly, Id2-deficient B cells have a preference for CSR over IgE because E2A induction of I ϵ -initiated transcripts is not antagonized (Sugai et al., 2003). Besides binding sites for STAT6 and E proteins, the I ϵ promoter binds other transcription factors, such as nuclear factor- κ B (NF- κ B), PU.1, Pax5, and bZIP family members (ATF, C/EBP, AP-1, see below) (Geha et al., 2003). All of these factors can activate germline ϵ transcripts, in synergy with IL-4.

Another cytokine, TGF- β 1, which promotes CSR to IgA and IgG2b, inhibits switching to IgE by inhibiting ϵ GLTs (Gauchat, Aversa, Gascan, & de Vries, 1992). Interestingly, the negative effect of TGF- β 1 on CSR to IgE could be explained by the induction of Id2 (Sugai et al., 2003). IFN- γ , which induces CSR to IgG2a, also inhibits ϵ GLTs and switching to IgE (Xu & Rothman, 1994).

Response to other factors is also different between I γ 1 and I ϵ promoters. *In vitro* systems have shown that CD40 signaling alone, in the absence of IL-4, could induce I γ 1 transcripts more efficiently than it does I ϵ transcripts (Mao & Stavnezer, 2001; Schultz et al., 1992; Warren & Berton, 1995). As all activated T cells express CD40L but only a fraction of them secrete IL-4, it is possible that many more B cells receive signals for IgG1 switching than for IgE switching. Another potentially important distinction lies in the activation of I γ 1 and I ϵ transcript by different bZIP transcription factors. While I γ 1 transcripts are activated by Activating Transcription Factor 2 (ATF-2/CREB2), I ϵ transcripts are activated by AP-1 (Mao & Stavnezer, 2001). Upon B cell activation, AP-1 upregulation is transient, whereas ATF-2 upregulation last for a much longer time. In combination with the absolute dependence of IgE CSR on IL-4, it is possible that the transient presence of AP-1 could contribute to the exquisite regulation of switching to IgE (Mao & Stavnezer, 2001).

Another bZIP transcription factor that could be differentially involved in CSR to IgG1 or IgE is Nuclear Factor, IL-3 regulated (NFIL3, also known as E4BP4). NFIL3 is essential for NK cell development, but is not expressed on resting T or B cells. Upon activation with IL-4, NFIL3 is rapidly and robustly induced. NFIL3-deficient mice displayed a three- to fivefold reduction in the IgE response, both total and antigen-specific, while the IgG1 response was unchanged (Kashiwada et al., 2010). The IgE defect was B cell intrinsic, as *in vitro* cultures of pure B cells stimulated with LPS + IL-4 resulted in the accumulation of about fourfold lower levels of IgE in the supernatants

than parallel cultures with WT B cells. In the same cultures, secreted levels of IgG1 were not changed between NFIL3-deficient B cells and WT B cells (Kashiwada et al., 2010). However, a parallel defect on IL-4 production by T cells was also reported in NFIL3-deficient mice, which would influence the IgE production in NFIL3-deficient mice (Kashiwada, Cassel, Colgan, & Rothman, 2011). As a consensus binding site for NFIL3 appears not to be present on the I ϵ promoter, the mechanisms by which NFIL3 influences IgE in a much more dramatic way than it does IgG1 are not totally clear. The affinity maturation of the IgE response in NFIL3-deficient mice was not evaluated.

In conclusion, CSR to IgE and to IgG1 share many key factors, but also display important differences that may explain the stringent regulation of IgE production compared to IgG1.



3. DIRECT AND SEQUENTIAL SWITCHING TO IgE

It has been reported that there are two paths to produce IgE antibodies from IgM in both humans and mice (Mandler, Finkelman, Levine, & Snapper, 1993; Siebenkotten, Esser, Wabl, & Radbruch, 1992). One is the Direct Switching pathway, where IgE cells are the product of direct S μ –S ϵ recombination; the other is the Sequential Switching pathway, a two-step process where S μ first recombines with one S γ , and then the hybrid switch region undergoes a second recombination with S ϵ . As will be discussed later, there are major biological implications associated with the direct versus sequential switching paths.

Due to the imprecise nature of CSR, some sequential switching events leave remnants of S γ sequences in the recombined S μ –S ϵ junctions and some do not (Fig. 4.1, S γ 1 remnants depicted as blue intervening sequences between S μ , depicted in green, and S ϵ , depicted in red). When S γ remnants are found, they represent a definitive indication of sequential switching in that allele (Yoshida et al., 1990), but the presence of S γ is not obligatory in sequentially switched S μ –S ϵ junctions, and absence of S γ remnants cannot be taken as an indication of direct S μ –S ϵ switching. In mice, sequential switching to IgE cannot involve S α , as the chromosomal segment containing the constant region of IgA (I α –S α –C α) is located downstream of that of IgE. In humans, the constant region of IgA1 is upstream of that of IgE, whereas the constant region of IgA2 is downstream. Thus, in mice, sequential switching to IgE can only involve the S γ regions, whereas in humans it can involve the S γ regions and S α 1.

Is there any preference in the S γ segments associated with sequential switching? Studies of a large number of S μ –S ϵ junctions showed that in mice undergoing type 2 responses, all remnants were from S γ 1 (Xiong et al., 2012). This tendency was expected as both I γ 1 and I ϵ promoters respond to IL-4, as discussed earlier, whereas other I γ promoters do not respond to IL-4. Mouse B cells activated *in vitro* with LPS + IL-4 also predominantly switched to IgE sequentially through IgG1, as addition of anti-IgG1 antibody to the cultures could reduce the amount of IgE by 70%, without affecting other isotypes such as IgG2a (Mandler et al., 1993). Once again, the presence of IL-4 in the cultures explains the bias toward possible IgG1 versus other IgG intermediates. In B cell cultures derived from mice that lacked the IgM switch region, IgE⁺ cells could be found at nearly normal levels (70%), and they were derived from CSR events involving S γ 1 (Zhang et al., 2010). However, databases contain mouse μ – ϵ switch region sequences that contain remnants other than S γ 1 (Dunnick, Hertz, Scappino, & Gritzmacher, 1993). To emphasize, sequential switching to IgE taking place *in vivo* during type 2 responses occurs only through IgG1 intermediates, often leaving S γ 1 remnants. Under different circumstances, other S γ segments can be found in S μ –S ϵ junctions.

As mentioned, evidence for sequential switching to IgE was also found in humans. For example, in a human B cell line, the switch to ϵ was preceded by a S μ –S γ 4 recombination (Mills, Thyphronitis, Finkelman, & Max, 1992). In human samples in which S μ –S ϵ junctions have S γ remnants, all four (S γ 1, S γ 2, S γ 3, and S γ 4) regions were found, with a predominance of S γ 1 remnants (Mills, Mitchell, Harindranath, & Max, 1995). Thus, the intervening S γ regions appear to be more diverse in humans than in mice; however, it is unknown whether IgE cells from humans undergoing strong type 2 responses would also have a dominating S γ region in their S μ –S ϵ junctions.

It was previously reported that IgG1⁺ cells had both ϵ germline (sterile) transcripts and ϵ switched transcripts, which are generated before and after the switch to C ϵ . Besides, we observed a substantial IgE response in TCR α ^{−/−} or Rag1^{−/−} mice that received pure IgG1⁺ cells and naïve T cells (Erazo et al., 2007). IgE antibodies made in these mice could only derive from the transferred IgG1⁺ cells that underwent sequential switching to IgE. Importantly, at all time points after *N. brasiliensis* infection of wild-type mice or antigen immunized transgenic mice, IgE⁺ cells were rare in germinal centers (GC). Since GCs are the place where affinity maturation takes place, it was unclear whether IgE antibodies could undergo affinity maturation. However, there was strong evidence of affinity maturation of

the IgE response, as both enhanced binding to antigen and affinity-enhancing somatic mutations were found in IgE⁺ cells (Erazo et al., 2007). Based on these observations, we hypothesized that sequential class switching through an IgG1 intermediate was a path to generate high affinity IgE antibodies. This model was recently demonstrated, as mice deficient in IgG1 were able to produce IgE at quasi-normal levels, but the IgE response lacked serological and mutational evidence of affinity maturation (Xiong et al., 2012).



4. IgE⁺ CELLS AND GERMINAL CENTERS

Many observations suggested that GC are, surprisingly, not favorable structures for IgE⁺ cells. Bcl6, which is highly expressed by germinal center cells, inhibits I ϵ GLTs by antagonizing STAT6-binding activity (Harris et al., 1999; Harris, Mostecky, & Rothman, 2005). IgG⁺IgE⁺ double positive cells were found in Bcl6-deficient B cells but were missing in Bcl6-Tg B cells *in vitro* (Kitayama et al., 2008). Bcl6-deficient mice have severely impaired germinal center formation and no affinity maturation, but they harbor an increased number of IgE⁺ cells (Ye et al., 1997). A recent study used intracytoplasmic IgE staining to show that a reduction of Bcl6 level led to the generation of IgE⁺ GC cells. The frequency went from a very low baseline (0.5%) to 1.8%. Inhibiting Bcl6 did not increase ϵ GLT equally in all GC cells. Instead, IgG1⁺ cells responded to a higher degree than non-IgG1 cells (Zhang, Makondo, & Marshall, 2012).

Besides Bcl6, deficiency in other GC-related molecules results in higher production of IgE. Deficiency of dedicator of cytokinesis 8 (Dock8) caused unstable germinal centers in mice (Randall et al., 2009) and hyper-IgE syndrome (HIES) in humans (Engelhardt et al., 2009; Zhang et al., 2009). IL-21 is a critical cytokine produced by T_{FH} cells; similarly to Bcl6 and Dock8, IL-21R-deficient mice have higher IgE and lower IgG1 level (Ozaki et al., 2002). Conversely, in the presence of IL-21, the production of IgE by LPS+IL-4-stimulated B cells *in vitro* was dramatically reduced while the production of IgG1 was increased. IL-21 inhibited the generation of IL-4 induced C ϵ GLTs, but not the phosphorylation of STAT6 (Suto et al., 2002). Further, sequential switching of IgG1⁺ cells to IgE was also inhibited by IL-21 (Erazo et al., 2007). Thus a major T_{FH}-produced cytokine in GC has a negative impact on IgE switching.

In light of these observations, it was perhaps not entirely unexpected that we found a low fraction of the IgE^+ cell in GC analyzed both by histology and the expression of GC markers by flow cytometry and qPCR on sorted cells. The paucity of IgE^+ cells in GC was found at all time points and in two unrelated experimental mouse models, the infection of wild-type mice with the nematode *N. brasiliensis*, and the immunization of TBmc (T + B monoclonal mice, which are mice with monoclonal repertoires of OVA-specific T cells and influenza HA-specific B cells) with the cognate antigen OVA-HA (Erazo et al., 2007). Instead, it was found that the majority of IgE^+ cells displayed a plasma cell phenotype, not a GC phenotype.

In the initial report on IgE^+ cells, a small but consistent fraction of IgE^+ cells was noticed. We wrote: “Although we found that the majority of IgE^+ cells have a PC phenotype, there is a low proportion of IgE^+ cells that are B220^+ and Syndecan^- . $\text{IgE}^+\text{B220}^+\text{Syn}^-$ cells are observed in both experimental systems that we studied, *Nippostrongylus*-infected WT mice ($15.6\% \pm 5.0\%$ of IgE^+ cells) and OVA-immunized TB monoclonal mice ($8.7\% \pm 3.3\%$). Gene expression studies on the $\text{IgE}^+\text{B220}^+\text{Syn}^-$ cells showed a GC pattern in both systems.” (Erazo et al., 2007). Thus, *Bcl6* expression, *IL-21*, and other factors do not completely prevent the development of some IgE^+ cells in GC. While the relatively low importance of IgE^+ GC in the IgE response still stands, the actual proportion of IgE^+ GC cells was underestimated in this study, largely because IgE^+ GC cells express low levels of *mIgE* (Section 6.2).

In summary, GC are not fully compatible with IgE^+ cells, but are perfectly compatible with IgG1^+ cells, which can sequentially switch to IgE . However, the observation of a low proportion of GC IgE^+ cells and the requirement of an IgG1 GC phase for the production of high affinity IgE antibodies have been recently challenged (see Section 6.2).



5. GERMINAL CENTER VERSUS PLASMA CELL FATES: *Bcl6* AND *Blimp1*

The data reviewed in the preceding section highlight the importance of understanding the factors that influence GC versus PC fate. The balance between GC and PC fate of B cells is determined by the differential expression of *Bcl6* and B lymphocyte-induced maturation protein (*Blimp1*). GC B cells express high levels of *Bcl6*, an antiapoptotic transcription factor (Cattoretti et al., 1995; Dent, Shaffer, Yu, Allman, & Staudt, 1997; Fukuda et al., 1997; Ye et al., 1997). *Bcl6* maintains the GC B cell

population and likely suppresses plasma cell differentiation by negatively regulating Blimp1 expression (Reljic, Wagner, Peakman, & Fearon, 2000; Shaffer et al., 2000), whereas Blimp1 directs cells to a plasma cell fate and negatively regulates Bcl6 expression (Kallies et al., 2004; Shaffer et al., 2002; Shapiro-Shelef et al., 2003; Turner, Mack, & Davis, 1994). These two opposing players, together with other factors such as IRF4 and FoxO, form a regulatory circuit to control GC or PC fate. Both expression of Blimp1 and downregulation of Bcl6 are required to achieve full PC differentiation (Diehl et al., 2008).

Different cytokines promote survival, proliferation, and differentiation of B cells. We mentioned IL-4, which is absolutely required for CSR to IgE (Section 2), and IL-21, which somehow blocks it (Section 4). IL-21 functions as an important regulator in the GC reaction and plasma cell differentiation (Linterman et al., 2010; Zotos et al., 2010). IL-21 is produced by T_{FH} T cells, which constitute an important T cell subset for GC formation and maintenance, and Ig-secretion in both mice and humans (Bryant et al., 2007; Chtanova et al., 2004). IL-21R is highly expressed on GC B cells (Barnett et al., 2012). In the absence of BCR signaling or cell contact with cognate T cells, IL-21 induces B cell death. The apoptotic effect of IL-21 on B cells *in vitro* is probably mediated through the proapoptotic molecule BIM (Jin, Carrio, Yu, & Malek, 2004; Mehta et al., 2003; Ozaki et al., 2004). However, in the presence of BCR signaling and the interaction with Th cells, IL-21 leads to differentiation of GC B cells to PC stage by inducing the expression of Blimp1 (Jin et al., 2004; Leonard & Spolski, 2005; Ozaki et al., 2004). For example, IL-21 transgenic mice show that IL-21 has positive effects on B cell number and antibody production especially IgM and IgG (Ozaki et al., 2004).

Recently, it was shown that asymmetric division caused an unequal distribution of Bcl6 and IL-21R in GC B cells, which may determine the different destinations of the daughter cells, and facilitate the survival and proliferation of the fittest cells with the highest affinity (Barnett et al., 2012). This could be a very important factor in the determination of GC versus PC cell fates.

The IL-21 receptor comprises IL-21R subunit and the common γ chain. Signaling through the IL-21R activates mainly STAT3 (Leonard & Spolski, 2005). STAT3, however, serves as a critical signaling molecule downstream of many cytokines that are involved in T cell and B cell development/functions. Besides IL-21, STAT3 is involved in the signaling of IL-6, IL-10, IL-11, and IL-23. Mutations in STAT3 have been found in patients

afflicted with the autosomal dominant hyper-IgE syndrome (AD-HIES) (Woellner et al., 2010; see below, Section 10). Although IL-21 and STAT3 have been ascribed many functions that are cell context dependent, conditional ablation of STAT3 on B cells revealed normal development and intact GC and memory B cell formation, but a severe impairment in PC differentiation, likely due to a defect in Blimp1 upregulation (Fornek et al., 2006).

Suppressor of cytokine signaling 3 (SOCS3) is a transcriptional target of STAT3 and also participates in the degradation of phospho-STAT3, establishing a negative feedback loop. Conditional deletion of SOCS3 in B cells resulted in a small but statistically significant reduction in antigen-specific GC cell numbers, which was accompanied by a late increase in antigen-specific IgE titers and enhanced affinity maturation of the IgE response (Jones, White, Robb, Alexander, & Tarlinton, 2011). As the effect of the absence of SOCS3 was evident only at late time points, and the IgG1 response was unchanged, it is possible that in the normal immune response, SOCS3 impairs the late sequential switching to IgE. These results offered yet another example of a negative correlation between GC and the IgE response.



6. STUDYING IgE RESPONSES *IN VIVO*

6.1. Flow cytometry analyses

One problem in studying IgE responses is that it is difficult to track IgE cells as they are rare *in vivo*, even though available anti-IgE antibodies are highly sensitive and specific. Further, CD23⁺ (FcεRII) or FcεRI⁺ cells can bind IgE and thus cause considerable false-positive signals in mice undergoing high IgE responses. CD23 is expressed on a wide range of cells including B cells, and binds IgE with low affinity (Ludin et al., 1987). Thus, in animals with high IgE levels, all B cells appear IgE-positive due to binding of IgE to CD23. FcεRI is mainly expressed on mast cells and basophils, not on B cells (Ravetch & Kinet, 1991), see also Section 1. For Immunohistochemistry, a mild and brief acid treatment method was developed in order to eliminate the low affinity binding of IgE to CD23 (Katona, Urban, & Finkelman, 1985; Katona, Urban, Scher, Kanellopoulos-Langevin, & Finkelman, 1983). Later, this method was adapted to flow cytometry: a 1-min incubation with acetate buffer, pH 4.0 or even pH 5.0, was sufficient to remove IgE bound to B cell via CD23 (Erazo et al., 2007). FcεRI staining and Fas staining further helped separate FcεRI⁺Fas⁺ IgE-producing B cells from mast cells/basophils that are FcεRI⁺Fas⁻ (Erazo et al., 2007).

These methods, combined with direct staining of surface IgE, enabled a precise detection of IgE-producing cells.

Other methods have also been developed to separate IgE-producing cells from IgE-covered cells. Intracytoplasmic IgE has been used to mark IgE-producing cells. A trypsin treatment was applied to effectively remove both membrane and receptor-bound IgE from the surface of B cells. Subsequent fixation and permeabilization of the cells allowed for specific staining of intracytoplasmic IgE, which exists only in IgE-producing B cells (Wesemann et al., 2011).

6.2. IgE reporter mice

The characterization of IgE reporter mice have been independently described by two labs (Talay et al., 2012; Yang, Sullivan, & Allen, 2012), by using GFP or Venus, a brighter derivative of YFP (Nagai et al., 2002). Both groups observed IgE⁺ cells in GC, but had discrepancies in the characterization of the populations, kinetics of IgE production, distribution of IgE plasma cells (PC), and memory response. Briefly, Yang et al. (2012) showed that GC IgE⁺ cells declined quickly, and had a different pattern of somatic mutations than IgE⁺ PC, indicating that IgE⁺ GC did not pass their mutation pattern to the IgE-producing PC. In sharp contrast, in Talay et al.'s (2012) paper, the GC IgE⁺ cells were long lived and made normal memory IgE⁺ cells.

As the endogenous IgE locus was manipulated in both reporter strains, the fidelity of these reporters needs to be evaluated. In Talay et al.'s study, the endogenous IgE gene was modified by inserting a human M1' domain right before the endogenous M1 exon splice acceptor site, which encodes the transmembrane domain of mIgE, and an IRES-GFP cassette was added after the M1–M2 segments to enable GFP coexpression. The resulting mouse human chimeric molecule is longer by 52 amino acids, and this long form does not occur in the mouse. Thus, the mouse strain used by Talay et al. reports the expression of a molecule that is not mouse IgE. The properties of this chimeric molecule compared to normal IgE were not determined. It is possible that signaling and antigen presentation capabilities of cells expressing the chimeric IgE are quite different from those displayed by cells expressing normal mouse IgE.

Most importantly, the polyadenylation signal of the membrane form, which controls IgE expression and RNA stability, was replaced in the construct utilized by Talay and colleagues (Brightbill et al., 2010). Of note, the

3' polyadenylation signal in mouse and human IgE genes is unusual, with three noncanonical hexamers, AGTAAA, AAGAAA, and ATTAAA. The result of this unusual configuration is an approximate 200–400:1 domination of sIgE over mIgE. A single mutation of the middle hexamer to the canonical AATAAA resulted in a 10-fold increase in the frequency of mIgE, the form expressed by IgE⁺ GC cells (Karnowski, Achatz-Straussberger, Klockenbusch, Achatz, & Lamers, 2006). Thus, minor manipulations of the polyadenylation site have the potential to affect the biology of IgE quite dramatically, by stabilizing the expression of mIgE in B cells that would normally die if the splice site was not manipulated (see Section 7). We cannot evaluate the distortion, if any, on the membrane to secreted IgE ratio in the mice described by Talay et al. (2012) because it was not reported. In summary, the main concern regarding the mice used by Talay et al. is that an artificial stabilization of the membrane form of IgE would result in IgG1-like IgE⁺ cells, and this appears to be the phenotype observed.

In Yang et al.'s study, the endogenous IgE gene was left intact until the final M2 IgE membrane exon, which was linked to Venus with a viral 2A peptide sequence (Yang et al., 2012). The authors studied the effect of the addition of Venus to the IgE locus on the proportion of membrane versus secreted IgE. Compared to the WT IgE allele, the reported allele displayed a two- to threefold increase of mIgE, and a reduction of secreted IgE, suggesting the splicing preference was shifted because of the Venus incorporation.

The authors observed Venus^{low} and Venus^{high} populations, with GC and PC markers, respectively (Yang et al., 2012). The low expression of mIgE in cells with a GC phenotype may have importance for the biology of these cells, as low membrane expression may limit their survival signals and the capacity to interiorize antigen and present it to T_H cells (Section 7). As previously reported by our lab, IgE⁺ plasmablasts and PC express mIgE, in contrast to what is known for other isotypes (Erazo et al., 2007). Interestingly, Yang et al. (2012) described that IgE-expressing PC expressed higher levels of mIgE than GC IgE cells.

Yang et al. studied the mutation profile of IgE and IgG1 cells in NP-KLH-immunized IgE-Venus reporter mice. Intriguingly, they found a significantly lower rate of total mutations and a lower rate of affinity-enhancing W33L mutations in IgE⁺ PC than in the IgE⁺ GC cells, which supposedly “feed” the PC compartment (as is the case for other isotypes). This was unexpected, unless the IgE PC are not derived from GC cells,

or unless only the early GC cells become PC and continue affinity maturation in the GC without further impact on the immune response. Importantly, Yang et al. observed that the Venus⁺ cells displaying the GC phenotype declined quickly over time, unlike IgG1⁺ GC cells in the same mice. These evidences suggest that there is a disconnect between the GC-like IgE⁺ cells and the real IgE-producing PC (Yang et al., 2012).

The brevity of GC stage was not due to swift differentiation into PC. While the number of IgE⁺ PC was greatly rescued in Bcl2 transgenic mice due to the antiapoptotic effect of Bcl2, the GC-like IgE⁺ population still retained its short-lived propensity. These observations suggest that most IgE⁺ PC were not GC derived. The authors reasoned that, possibly, IgE⁺ GC B cells prematurely differentiated into early PC, and this would both reduce the affinity of the PC pool and diminish the size of the pool of IgE⁺ GC B cells capable of continuing affinity maturation. However, the data are also entirely consistent with the sequential switching μ - γ 1- ϵ reported by our laboratory as essential for the affinity maturation of the IgE response. In our model, the mutated IgE⁺ PC would derive largely from mutated IgG1⁺ GC cells, not from the IgE⁺ GC cells, thus explaining the disconnect that Yang et al. observed between IgE⁺ GC cells and IgE⁺ PC (Xiong et al., 2012). In any case, GC-like IgE⁺ cells are not an efficient pool from which to select high affinity IgE clones.

Taken together, from the three manuscripts (Erazo et al., 2007; Talay et al., 2012; Yang et al., 2012) one can conclude that the percentage of B220^{high} IgE⁺ GC cells may have been underestimated in our initial report. The degree of the underestimation is, however, unclear, as one reporter construct has a problem with membrane to secreted IgE ratio (Yang et al., 2012), and the other construct, which is much more likely to have bigger distortions, was not tested (Talay et al., 2012). Regarding the IgE⁺ GC cells, their low mIgE expression, their transient nature, and the different mutation rate of IgE⁺ PC suggest the possibility that these cells represent a terminal state that does not play a significant role in the affinity maturation of IgE antibodies. Their role, thus, remains obscure. Another conclusion, shared by two of the three reports, is the high importance of IgE⁺ PC and how quickly they take over the IgE response. Further, the data by Yang et al. is in no way inconsistent with the sequential switching model and its importance for affinity maturation of the IgE response. In the manuscript by Talay et al., no evaluation was made of the affinity maturation of the IgE response.



7. IgE CYTOPLASMIC TAIL AND GERMINAL CENTERS

One of the possible explanations for the lower than expected frequency of B cells at GC could be a weaker signaling by IgE⁺ B cells compared to B cells expressing other isotypes. In the competitive environment of GC, it is possible that cells switching to any of the IgGs have a survival advantage over cells that switch to IgE. It has been established that one of the key survival advantages for GC B cells is the amount of T cell help that they receive (Tarlinton, 2008). It has also been recognized that the cytoplasmic domain of immunoglobulins is essential for antigen presentation to T cells. Essentially, antigen-driven interiorization of the BCR is the first step of the process by which B cells present antigen to T cells. It has been shown that the domains required for B cell activation and antigen presentation are different. For example, phosphorylation of the ITAM in CD79 is not required for antigen presentation, but is required for BCR-mediated B cell activation (Tarlinton, 1997). The cytoplasmic tail of immunoglobulins is required for interiorization and antigen presentation. Using an iodo-NP (NIP)-specific $\gamma 2a$ chain, Weiser et al. demonstrated that the motif YxxM, which is present in the cytoplasmic tail of all IgGs, was essential for BCR internalization and antigen presentation to T cells (Weiser, Muller, Braun, & Reth, 1997). Similar findings were made for human IgG1 regarding the importance of the Y in the YxxM motif (Knight, Lucocq, Prescott, Ponnambalam, & Watts, 1997).

The cytoplasmic tail of mouse immunoglobulins varies with the immunoglobulin class. However, the class-specific cytoplasmic tail sequences are highly conserved between mice and humans. IgM and IgD molecules have a very short tail composed of the amino acids KVK. All IgGs have 28 amino acid cytoplasmic tails, which start with KVK and display high homology through the remaining 25 amino acids (Fig. 4.2). IgA has a 14-amino acid tail, which lacks the KVK motif.

Interestingly, the cytoplasmic tail of murine IgE starts with KVK and has the same 28-amino acid length as all IgG tails. The KVK motif is missing from human IgE, but the length is conserved (Fig. 4.2). Both murine and human IgE cytoplasmic tails have homology with themselves but little homology with murine or human IgG tails, and lack the key YxxM motif present in all IgGs; instead, both mouse and human IgE tails have an YxNI motif (Fig. 4.2). Whether the YxNI motif allows for some degree of antigen presentation is unclear.

Mouse IgG1	K	V	K	W	I	F	S	S	V	V	E	L	K	Q	T	L	V	P	E	Y	K	N	M	I	G	Q	A	P
Mouse IgE	K	V	K	W	V	L	S	T	P	M	Q	D	T	P	Q	T	F	Q	D	Y	A	N	I	L	Q	T	R	A
Human IgE	H	V	Q	R	F	L	S	A	T	R	Q	G	R	P	Q	T	S	L	D	Y	T	N	V	L	Q	P	H	A
Mouse IgE	K	V	K	W	V	L	S	T	P	M	Q	D	T	P	Q	T	F	Q	D	Y	A	N	I	L	Q	T	R	A
Human IgG1	K	V	K	W	I	F	S	S	V	V	D	L	K	Q	T	I	I	P	D	Y	R	N	M	I	G	Q	G	A
Mouse IgG1	K	V	K	W	I	F	S	S	V	V	E	L	K	Q	T	L	V	P	E	Y	K	N	M	I	G	Q	A	P

Figure 4.2 Alignment of the cytoplasmic domains of human and mouse IgE and IgG1. The KVK and the YxxM motifs are spaced from the other parts of the domain to facilitate their identification. The Y and M of the YxxM motif are depicted in boldface font. Amino acid sequences are aligned in pairs, as indicated. Shaded amino acids are identical in a particular pair. Note that human and mouse IgG1 molecules display high levels of homology throughout the cytoplasmic domain; in contrast, there is little homology between IgE and IgG1 in both species.

More than a decade ago, the Lamers’ group in Germany made a deletion of IgE cytoplasmic tail. From the IgE tail, they kept the membrane-proximal amino acids KVK. The KVK residues are essential to keep IgM in the membrane, but are not involved in signaling. Indeed, cells with a deletion of the cytoplasmic tail below the KVK were capable of expressing surface IgE. Serum IgE levels in unmanipulated mice were reduced by 50%, and, similarly, upon immunization with TNP-OVA, serum titers were reduced by 50–80%. A potent secondary response against *N. brasiliensis* infection was present in mice with the IgE cytoplasmic deleted, although it was reduced by about 50% compared to WT mice (Achatz, Nitschke, & Lamers, 1997).

In contrast to the relatively mild effect of the absence of the IgE cytoplasmic tail, the equivalent manipulation in IgG1⁺ cells resulted in a 24-fold reduction in serum IgG1 and at least one log reduction in affinity of anti-NP antibodies upon immunization with NP-CG (Kaisho, Schwenk, & Rajewsky, 1997). Mice defective in IgG1 cytoplasmic tail switched to IgG1 in normal proportions *in vitro* upon stimulation with LPS and IL-4, indicating that the deletion of the tail was, most likely, affecting antigen presentation to T cells.

Regarding the mild effect of the IgE tail deletion on IgE responses, it is possible that, in the absence of the YxxM cytoplasmic tail motif in IgE, CD79 could be part of BCR interiorization and antigen presentation, compensating for the lack of cytoplasmic tail. However, another interpretation is that the remaining IgE did not require a GC phase, in which T cell help is essential. It is possible that IgE did not require a GC phase because the GC phase was carried out by IgG1⁺ cells. In this scenario, T cell help would be obtained by IgG1-mediated antigen interiorization, with IgE-switched cells

becoming much less dependent on antigen presentation to T cells than IgG1⁺ cells. Thus, the differences in the IgG1 and IgE cytoplasmic tails could be part of the explanation of why IgE cells do poorly in the competitive GC environment.

If IgE⁺ cells were rare and unstable in GC due to their disadvantaged cytoplasmic tail, addition of an IgG1 cytoplasmic tail would rescue the GC phenotype of IgE⁺ cells. In order to test this hypothesis more directly, cells coexpressing IgG1 and IgE in their surfaces were studied (Erazo et al., 2007). As there is no allelic exclusion in CSR, these cells expressing two isotypes are possible if the VDJ allelic exclusion, which is very powerful, is not in play. To accomplish this, we made homozygous heavy chain knockin mice from TBmc mice, in which the same productively rearranged VDJ chain was expressed by both alleles. Immunization of these heavy chain homozygous TBmc mice with OVA-HA resulted in a low percentage of IgG1⁺IgE⁺ cells (<0.05%). These cells were sorted, and the dual expressing IgG1/IgE nature of these rare cells was confirmed by qPCR of mature transcripts. Characterization of these cells indicated that they lacked GC markers and, instead, they had plasmablast characteristics typical of single-expressing IgE⁺ cells. This result indicates that an IgG1 cytoplasmic tail is not sufficient to rescue the GC phenotype of IgE⁺ cells.



8. IS THERE A MEMORY IgE⁺ CELL?

It is generally believed that long-lived PC derive from a persistent GC reaction (Radbruch et al., 2006), and that many long-lived memory cells reside in the bone marrow (BM). While memory IgE responses can be readily demonstrated, it has not been conclusively shown that they are carried out by memory IgE⁺ cells. The alternative is that, like the high affinity IgE response, the memory IgE response is carried out by IgG1⁺ cells, which, upon receiving the appropriate signals, subsequently switch to IgE. Yang et al. found that the IgE⁺ cells were predisposed to more readily differentiate into short-lived PC, and failed to migrate to BM effectively (Yang et al., 2012), a similar finding as described by our lab (Erazo et al., 2007). As indicated above, studying IgE⁺ cells *in vivo* is not particularly easy, and finding memory cells among them is even harder. We could not detect IgE⁺ cells with a memory phenotype, as most IgE⁺ cells acquired a plasmablast phenotype. These two studies did not report the presence of IgE⁺ memory cells, but were not conclusive in proving their absence, which is more difficult.

In contrast, Talay et al. described a clear memory IgE (GFP⁺) population that expressed CD38 highly. However, as described above, the way Talay's et al. reporter mice were generated is likely to have resulted in an abnormally increased membrane to secreted IgE ratio compared to IgE⁺ cells from wild-type mice, making these IgE⁺ cells behave more like IgG1⁺ cells. Further, in the mice used by Talay et al., the secondary IgE response upon *N. brasiliensis* reinfection was neither as fast nor as strong as typical memory IgE responses. The peak of the secondary response occurred only 2 weeks after reinfection and reached only 2.5 µg of IgE/ml. In contrast, a prior publication using *N. brasiliensis* reinfection showed the peak of IgE occurring 1 week after reinfection and reaching approximately 150 µg of IgE/ml (Achatz et al., 1997).

It needs to be noted that in Talay's et al. and Yang's et al. studies, C57BL/6 (B6) mice were used. The IL-4 and IgE response in B6 mice is weak. IgE responses reported by Talay et al. and Yang et al. are 1–10% of those reported in BALB/c background mice (Erazo et al., 2007; Talay et al., 2012; Xiong et al., 2012; Yang et al., 2012). B6 is not the strain most used for the study T_H2 responses (Drazen, Arm, & Austen, 1996) because it produces little IL-4. The difference between genetic backgrounds in terms of IgE GC and memory responses needs to be addressed more clearly in the future.

Our studies showed that the frequency of sequential switching increased with enhancing affinity of IgE antibody, and that sequentially switched IgE had a higher mutation level in both the switch regions and the VDJ region. In a loss-of-function model, IgG1-deficient mice had impaired IgE affinity maturation. Remarkably, these IgE antibodies, which were all directly switched from IgM to IgE, lacked the specific somatic hypermutations that confer the antibody high affinity to its antigen (Xiong et al., 2012). Although this study did not specifically address the question of IgE⁺ memory cells, our results suggested that memory IgG1⁺ cells could provide an efficient way to generate high affinity IgE. We proposed that the majority of the IgE⁺ PC in the secondary response did not derive from putative memory IgE⁺ cells, but rather, were produced by IgG1⁺ cells through a swift second switching (γ1-ε). Otherwise, it would have not been possible to explain the role of IgG1 in the affinity maturation of the IgE response and the increased affinity (i.e., memory) upon repeated immunizations.

If IgG1⁺ cells originate the IgE memory response, in which location does this second switch take place, and under which conditions? GC IgG1⁺ cells express Cε switched transcripts (Iμ-Cε) (Erazo et al., 2007). The presence of these transient transcripts indicates that CSR to IgE took place within these

GC cells sorted by membrane IgG1 expression. It is possible that the switched transcripts derive from the other chromosome, not recombined to $\text{S}\gamma 1$, but it is also possible that, at least in some of these cells, membrane IgG1 expression remained for some time after the $\text{C}\gamma 1$ gene was deleted by the second CSR event on the same chromosome. Therefore, a GC origin of IgE^+ cells is not excluded by the data. We previously showed that, *in vitro*, sequential switching of IgG1^+ memory cells to IgE requires IL-4. This was not the case for IgG1, which was produced only upon stimulation with anti-CD40 (Erazo et al., 2007). Two observations are relevant to this point. One, besides IL-21, T_{FH} cells also produce large amounts of IL-4, and at least some IL-4^+ T_{FH} cells coexpress IL-21 (Reinhardt, Liang, & Locksley, 2009). As indicated above (Section 2), IL-21 blocks CSR to IgE while IL-4 activates it. Could T_{FH} cells be the source of IL-4 required for IgG1^+ cells to undergo the second switching event? $\text{T}_{\text{H}2}$ cells outside GC could also do it, without the “mixed signals” delivered by IL-21. Second, it has now been shown by two groups that isotype-switched memory B cells do not go to GC or originate new GC upon reimmunization (Dogan et al., 2009; Pape, Taylor, Maul, Gearhart, & Jenkins, 2011). Thus, if IgG1^+ memory cells are to provide the memory IgE response, the second switch is likely to occur outside GC, and not be driven by T_{FH} cells. These are key questions that still remain unanswered.



9. DIRECT AND SEQUENTIAL SWITCHING TO IgE IN IMMATURE AND MATURE B CELLS

CSR not only escapes allelic exclusion rules, but it can also occur in immature B cells. A recent paper found that the B cell developmental state has an impact on the IgE response by regulating direct versus sequential switching to IgE (Wesemann et al., 2011). By comparing IgE and IgG1 switching of early B cell lineage/immature B cells (IBC) with that of mature splenic B cells, the authors showed that the IBC cultures underwent more CSR to IgE than the mature B cell culture. IBC had a lower level of $\text{I}\gamma 1$ transcripts and consequently lower IgG1 production, and faster switching to IgE *in vitro* than mature spleen B cells. These phenomena were all directly correlated with an increased proportion of direct μ – ϵ switch in IBC compared to mature B cells. Similar tendency, although less marked, was observed in B cells from young mice compared to adult mice. These results provided a possible explanation for the higher levels of IgE in children compared to adults, and why many HIESs are linked with different primary immunodeficiencies that impair lymphocyte development and function (see Section 10).

Increased IgE CSR was observed in mature B cells that cannot switch to IgG1 because of deletion of $\text{S}\gamma 1$ (Misaghi et al., 2010). It has been shown that trimethylation of K4 in histone H3 in I promoters correlated with increased GLT. There was a decrease of H3K4me3 at $\text{I}\gamma 1$ in IBC compared to mature B cells, consistent with the lower proportion of CSR to $\text{S}\gamma 1$ in IBC. There was no significant change at $\text{I}\epsilon$. In addition, IBC had lower levels of phospho-STAT6, which should affect CSR to both $\text{S}\gamma 1$ and $\text{S}\epsilon$ (Wesemann et al., 2011). These results suggested that a competition for the chromatin modification and transcriptional machineries could determine the preference for $\text{I}\epsilon$ or $\text{I}\gamma 1$ -initiated GLT. According to the simplified competition model, decreased IL-4 levels, which in turn, affect phospho-STAT6 levels, would favor IgE production, because STAT6 binds with higher affinity to $\text{I}\epsilon$ than to $\text{I}\gamma 1$ (Section 2). However, moderately decreasing the levels of IL-4 reduced both IgG1 and IgE production, indicating a more complicated correlation than a simple competition between IgE and IgG1 production. Sequential class switching from IgG1 to IgE provides a potential explanation for these observations. If a considerable proportion of IgE was produced from IgG1^+ cells, the IgG1 and IgE generation would be positively related to a large extent.



10. HUMAN IMMUNODEFICIENCIES WITH HIGH IgE PRODUCTION

HIESs can be mostly classified according to the autosomal dominant or recessive nature of the abnormalities. AD-HIES is generally caused by STAT3 mutations, and is also known as Job's syndrome, for the cutaneous abscesses. A number of recessive mutations also result in hyper-IgE, predominantly DOCK8 or Tyk2 mutations. Di George syndrome, Omen's syndrome, and Wiskott-Aldrich syndrome also have been associated with hyper-IgE.

Besides a very high serum IgE level, a common feature of these HIESs is a T cell deficiency resulting in overt T lymphopenia, or a functional impairment in the T cell compartment. The paradox of a preferential $\text{T}_{\text{H}2}$ differentiation during lymphopenic states was clearly demonstrated (Milner, Ward, Keane-Myers, & Paul, 2007), but remains largely unexplained. In the case of dominant STAT3 mutations, these patients also have decreased $\text{T}_{\text{H}17}$ cells, which explain the mucocutaneous candidiasis and other infections (Milner et al., 2008). However, the connection between decreased $\text{T}_{\text{H}17}$ cells and increased $\text{T}_{\text{H}2}$ cells is not clear. It has been proposed that

T_H17 and Treg cells have a reciprocal development pathway (Bettelli et al., 2006), with a defect in T_H17 generation bringing the possibility of an increased Treg population. However, it is highly unlikely that any of the manifestations of HIES are due to increased Treg cells. To the contrary, Treg cells control both T_H17 and T_H2 cells, and it has been shown in mice that absence of Treg cells results in hyper-IgE (Curotto de Lafaille et al., 2001).

AD-HIES is caused by dominant negative STAT3 mutations, which are located in the DNA binding domain or the SH2 domains (Woellner et al., 2010). These patients are afflicted by recurrent *Staphylococcus aureus* infections in the skin and lungs, as well as mucocutaneous candidiasis. Very high IgE levels are reached.

The pathogenesis of AD-HIES is incompletely understood. STAT3 is a molecule involved in the signaling of many diverse cytokines such as the common γ c family of cytokines, the IL-10 family of cytokines, the gp130 family of cytokines, and more. STAT3 is involved in IL-6 and IL-23 signaling, which are involved in T_H17 cell generation, and this defect may explain the susceptibility to infections, as discussed above. In contrast, IL-10, whose signaling also involves STAT3, is an immunoregulatory cytokine, and its defect may explain the loss of immune regulation of IgE in AD-HIES. STAT3 is also involved in IL-21 signaling. IL-21R-deficient mice have augmented IgE levels (Ozaki et al., 2002), and IL-21 blocks IgG1 sequential switching to IgE (Erazo et al., 2007). Thus, STAT3-dominant negative mutations can act at multiple levels to explain the symptomatology of AD-HIES patients.

From the perspective of this review, the most remarkable aspect of AD-HIES patients is the extremely high level of IgE, which is not accompanied by allergic manifestations other than skin disorders. Asthma, allergies, and anaphylaxis are rare (Freeman & Holland, 2008, 2009).

As indicated above, we postulate a model whereby class switching to IgE follows a sequential route via an IgG intermediate in order to generate high affinity, anaphylactogenic antibodies (Xiong et al., 2012). A clear prediction of the sequential switching model is that most IgE⁺ cells in AD-HIES patients are not the result of affinity maturation. If this was the case, with the high IgE levels that are attained, cross-linking with minute amounts of environmental antigens would trigger massive allergic reactions.

The situation is different with patients with DOCK8 mutations. Not all patients with autosomal recessive DOCK8 deficiency have hyper-IgE (Su, Jing, & Zhang, 2011). Thus, comparison of DOCK8 patients with high IgE and normal IgE could be revealing. Another interesting difference between DOCK8 mutations and AD-HIES patients is that patients with

DOCK8 mutations frequently develop symptoms of atopic disease outside the skin, such as asthma, severe allergies, and anaphylaxis. Comparison of the S μ -S ϵ switch regions of DOCK8 patients with those of AD-HIES (Job's syndrome) patients should be informative, as it would indicate the affinity maturation pathways of IgE-producing cells in these patients.



11. CONCLUDING REMARKS

Over the past several years, it has become increasingly evident that the IgE response has some unique features. While in our early work, we described some IgE⁺ cells with GC characteristics during immune responses of wild-type mice to a nematode parasite, by far the most salient feature of the IgE response compared to IgG responses was, in fact, how PC-centric the IgE response was found to be. Mice defective in IgG1 display an impaired affinity maturation of the IgE response, which indicates that the GC selection of high affinity IgE⁺ cells is left for IgG1 cells that are subsequently driven to switch to IgE, under conditions that remain very poorly understood. Further, it is not yet known whether the same principles apply to the human high-affinity IgE response, although at this moment there are no reasons to think that they would not apply. This issue needs to be clarified in the near future.

It is even unclear whether bona fide memory IgE cells exist, or IgG1 memory cells sequentially switch to IgE, thus providing the memory IgE response. In neither wild-type mice infected with a nematode parasite nor immunized transgenic mice with a defined B cell repertoire was a defined population of memory IgE⁺ cells observed. This is another important focus for future studies.

Regarding reactions such as anaphylaxis, the affinity maturation of IgE antibodies could mean the difference between life and death, so it is apparent that discovering the factors and conditions that drive the sequential switching of some IgG1⁺ cells to IgE will be a major step forward.

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