

REVIEW

Regulation of the humoral type 2 immune response against allergens and helminths

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The type 2 immune response is associated with helminth infections and allergic inflammation where antibody production of the IgG1 and IgE isotypes can elicit protective or proinflammatory functions. Studies over the past few years revealed important new insights regarding the regulatory mechanisms orchestrating the humoral type 2 immune response. This includes investigations on B-cell extrinsic signals, such as IL-4 and IL-21, derived from different T-helper cell subsets or discovery of new follicular helper T cells with regulatory or IgE-promoting activities. In addition, studies on B-cell intrinsic factors required for germinal center formation and class switch recombination, including the transcription factors STAT3, STAT6, and BCL-6, led to a better understanding of these processes in type 2 immune responses. Here, we review the current understanding of mechanisms controlling humoral type 2 immunity in vivo including the generation of IgE-producing plasma cells and the memory IgE response.

Keywords: Allergy · Germinal center · Helminths · IgE · STAT6

Introduction

Type 2 immune responses are frequently elicited by infections with larger parasites, such as helminths, allergens, and venom toxins. Hallmarks of type 2 immunity include an increase in numbers of IL-4, IL-5, and IL-13-producing effector cells such as Th2 cells, type 2 innate lymphoid cells (ILC2s), NKT cells, eosinophils, basophils and mast cells, differentiation of alternatively activated macrophages, goblet cell hyperplasia, smooth muscle cell activity, and elevated serum levels of IgE and IgG1. The cytokines IL-4 and IL-13 play a key role in type 2 immune responses and bind to heterodimeric receptors composed of either the IL-4R α chain together with the common gamma (γ) chain (type I IL-4 receptor, binds only IL-4) or the IL-4R α together with the IL-13R α 1 chain (type II IL-4 receptor, binds IL-4 and IL-13) [1, 2]. IL-13 further binds to IL-13R α 2, which can be expressed as transmembrane or soluble IL-13 receptor. The type I receptor is mainly expressed on hematopoietic cells, while the type II receptor is expressed on

nonhematopoietic cells but also on B cells [3]. Both receptors activate the transcription factor STAT6, which plays an important role for the humoral type 2 immune response by promoting GC formation and differentiation of IgE-producing plasma cells (PCs). IgE-sensitized mast cells and basophils can contribute to protective immunity against helminths but also elicit allergic inflammation in response to allergens.

In this review, we discuss recent findings regarding the cellular and molecular mechanisms that control the humoral type 2 immune response at different levels, including the pre-GC phase, formation of GCs, role of Th2, and T-follicular helper (Tfh) cells and the generation of memory B cells and IgE-producing PCs.

Initiation of the GC response

The GC is a microenvironment generated during an immune response and localized within the B-cell zone in secondary lymphoid organs. GCs consist mainly of activated B cells, follicular dendritic cells (FDC) on the surface of which B cells see their antigens and Tfh cells that provide important differentiation and

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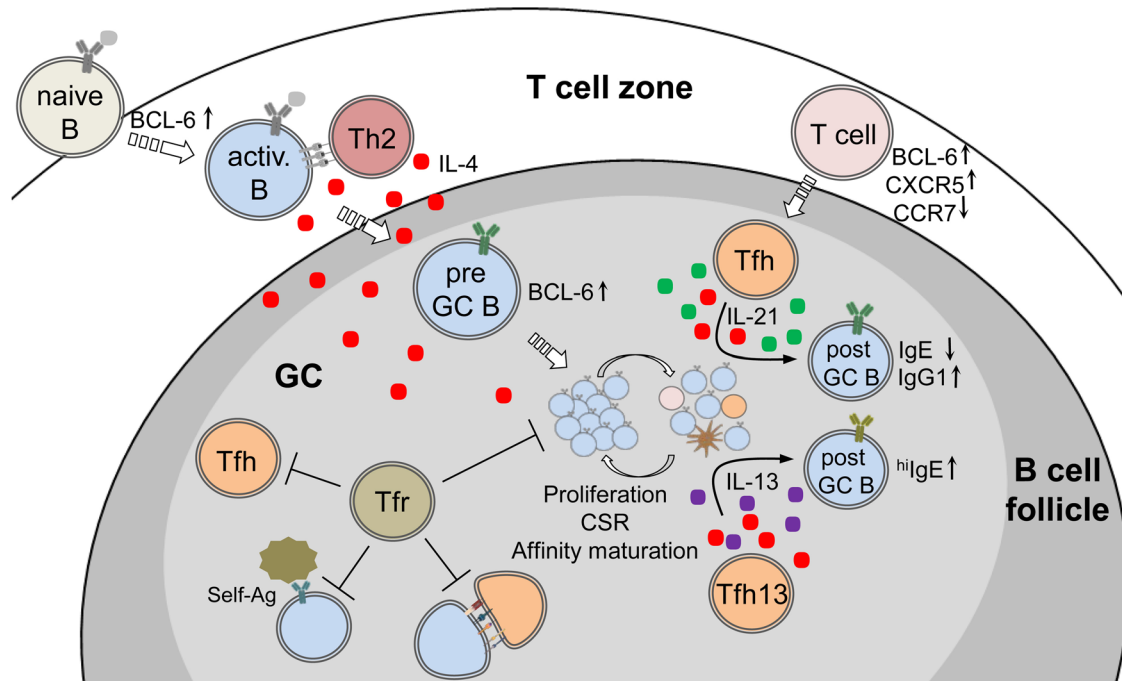


Figure 1. Signaling and regulation of the humoral type 2 immune response in the germinal center. Naïve B cells recognizing their cognate antigen upregulate BCL-6. In the T-B cell border, the activated B cells receive further stimulation by T-helper cells (Th) and IL-4 further promotes BCL-6 upregulation and entry into the GC. Furthermore, IL-4 by Th2 cells located in the T-cell zone permeates the whole B-cell follicle providing IL-4 signaling to all GC B cells. In the GC reaction, B cells participate in a repetitive cycle of proliferation, affinity maturation, class-switch recombination (CSR), and selection. This is dependent on signaling via IL-4, IL-21, and IL-13 provided by follicular helper T cells (Tfh). Tfh cells are generated from T cells that receive sufficient stimulus and upregulate BCL-6 and CXCR5, and downregulate CCR7. IL-21 further promotes CSR to IgG1 and inhibits IgE whereas IL-13 can lead to production of high affinity IgE (^{hi} IgE) with anaphylactic potential. Further regulation is provided by regulatory follicular T cells (Tfr) which directly suppress Tfh cells as well as autoreactive B cells recognizing self-antigen. Tfr cells also control the interaction of Tfh and B cells and limit the expansion of the GC.

survival signals to GC B cells. A GC is initiated after naïve B cells encounter their cognate antigen, which happens either outside of the B-cell follicle, in the subcapsular sinus of lymph nodes, in the submarginal sinus of the spleen, or directly in the B-cell follicle. B cells then upregulate the chemokine receptor CCR7 that guides them to the border between B-cell follicle and T-cell zone where they present internalized antigens to T-helper cells (Th) and receive further activating signals, mainly by CD40-CD40L interaction and cytokines. Such activated B cells downregulate expression of the chemotactic receptor EBI-2 (GPR183) and migrate into the B-cell follicle to establish the GC where they can undergo Ig class switch recombination (CSR) and acquire somatic mutations in Ig genes which leads to affinity maturation by competitive selection within the GC structure [4, 5] (Fig. 1).

CSR and acquisition of somatic mutations are both dependent on the enzyme Activation-induced cytidine deaminase (AID) which shows highest expression in GC B cells [6]. CSR inside GCs has been reported to happen after the onset of somatic mutations [7]. However, a recent study in mice revealed that CSR mainly occurs before B cells enter the GC [8]. Others have shown that affinity maturation and CSR can also take place in a GC-independent manner under certain conditions [9, 10]. For example, mice lacking Treg showed elevated levels of IgE against allergens and this IgE production occurred independently of GCs

[11]. Furthermore, patients with CD40L-deficiency were found to contain IgE + CD27⁺ B cells with few SHM suggesting a GC-independent origin [12].

Role of T cells and cytokines for GC formation and IgE production in mice during type 2 immunity

Tfh cells are characterized by their localization in GCs and expression of BCL-6, PD-1, CXCR5, CD40L, ICOS, and IL-21. They can originate from Th1, Th2, or Th17 cells and keep expression of the respective cytokines [13]. Hence, in type 2 immune responses Tfh cells can express both IL-21 and IL-4, and both cytokines play an important role for efficient GC formation. Mechanistically, they inhibit the degradation of the key transcriptional repressor BCL-6 which is required for the GC B-cell differentiation program [14]. In addition, IL-4-induced STAT6 activation promotes transcription of BCL-6 [14]. Although IL-4 and IL-21 can act on many cell types, several studies have shown that efficient GC formation requires direct recognition of these cytokines by B cells [15–17]. Kinetic studies further revealed that IL-21 expression precedes IL-4 expression in Tfh, before their migration into the B-cell follicle, indicating that IL-21 in contrast to IL-4 has a dominant role at the pre-GC B-cell stage [18]. However, others have shown that

an early wave of NKT cell-derived IL-4 also plays an important role for GC seeding by B cells during viral infections [19]. Besides their common activities, such as promoting B-cell proliferation and survival, IL-21 and IL-4 also appear to have opposite functions regarding IgE CSR and generation of IgE-producing PCs.

IL-21 promotes B-cell proliferation and plasma blast differentiation by binding to the IL-21 receptor on B cells which is associated mainly with STAT3 for signal transduction and regulation of gene expression. IL-21 further inhibits IgE production while promoting the IgG1 response in a STAT3-dependent manner [20, 21]. Consistent with these findings, IL-21 and IL-21R-deficient mice develop spontaneous hyper-IgE phenotypes [22]. In humans, a dominant negative mutation of STAT3 results in hyper-IgE syndrome [23]. This indicates that Tfh-derived IL-21 increases the threshold for IgE CSR in GCs.

Tfh cells were further found to be the major source of IL-4 in reactive lymph nodes in type 2 immune responses against *Leishmania major* or different helminth infections [24–26]. However, this does not necessarily prove that they are the critical source of IL-4 for GC formation and IgE CSR. In fact, IL-4/IL-13 expression by CXCR5-deficient T cells that could not enter GCs was sufficient for a normal GC and IgE response [17]. This indicates that IL-4 from Th2 or pre-Tfh cells outside GCs plays a dominant role. Furthermore, it was shown that the majority of B cells in reactive lymph nodes of helminth-infected mice contain phosphorylated STAT6 indicating that IL-4 can diffuse through an entire LN [27]. Others have found that deletion of IL-4 in Tfh cells results in a diminished Th2 and IgE response to helminth infection [28]. It was further reported that Tfh-deficient mice develop impaired GCs and a poor IgE/IgG1 response in a mouse model of allergic lung inflammation demonstrating the general requirement of Tfh cells but not necessarily IL-4 production by them [29]. Studies with selective and inducible deletion of IL-4 in Tfh cells would be helpful to resolve this issue.

A recent study reported the identification of a small subset of Tfh cells in mouse and man that expresses the transcription factor GATA3 and high levels of IL-13 in addition to IL-4 and IL-5 [30]. These Tfh13 cells were only found after repeated challenge of mice with various allergens but not after primary infection with the helminth *Nippostrongylus brasiliensis*. Interestingly, Tfh13 cells promoted the differentiation of PCs producing high-affinity IgE antibodies that caused anaphylactic response upon antigen challenge [30]. In vitro stimulation of B cells with anti-CD40, IL-4, and IL-13 resulted in more IgE+ PCs as compared to cultures without IL-13. Further investigations are required to understand how IL-13 signaling in B cells or PCs promotes the generation of high-affinity anaphylactic IgE antibodies and whether this process is also relevant in the human immune system. A better understanding of this pathway could then lead to development of new therapeutic interventions to prevent formation of PCs producing anaphylactic IgE antibodies.

Several efforts are being made to inhibit Tfh functions in allergic conditions of humans. In a recent clinical study, it was observed that circulating CXCR5+ T cells which show a phenotype similar to Tfh cells in GCs were increased in number

in patients with allergic asthma [31]. Those cells provide help to B cells like conventional Tfh cells and cause an increase of serum IgE. Another study observed increased Tfh cell numbers with more IL-4 secretion in tonsils of children sensitized to house dust mite allergens [32]. This marks Tfh cells as promising candidate for therapy with the ICOS/ICOS-L pathway being one potential target. In fact, treatment of mice with anti-ICOS-L antibodies ameliorated established allergy in the house dust mite model [33]. Another study in humanized mice demonstrated that NK cells expressing a chimeric antigen receptor against human PD-1 successfully removed Tfh cells without affecting other T-cell populations showing a promising future for treatment against Tfh-mediated diseases [34].

Regulation of the GC and IgE responses by follicular regulatory T (Tfr) cells

Tregs are an important part of the immune system and are tasked with inhibition of self-reactive T and B cells [35, 36]. However, they also have further functions beyond protection against autoimmunity. Tregs are also present in the GC and constitute a distinct subset named follicular regulatory T (Tfr) cells. The function of these cells is to maintain and control the overall GC reaction. Deleting Tfr cells leads to increased GC size and GC B-cell proliferation but also causes impaired affinity maturation and increased autoimmunity [37, 38]. Tfr cells can either directly confer inhibitory signals to B cells or modulate the GC response indirectly via interfering with interactions between Tfh cells and B cells [36, 39]. By these mechanisms Tfr cells can generally fine tune the GC response (Fig. 1).

Tfr cells also play a critical role for controlling IgE responses. It was shown in mice that expression of the transcriptional repressor Blimp-1 in Tfr cells is required for prevention of spontaneous autoantibody production including self-reactive IgE [35]. Blimp-1 promoted the Tfr phenotype by maintaining expression of Foxp3, activating the STAT5 signaling pathway, and controlling CXCR5/CCR7 expression for homing of Tfr cells into the GC. Tfr cells also regulate the immune response to allergens as shown in a mouse model of house dust mite allergy where Tfr cells modulated the activity of Tfh cells [38]. In addition, Tfr cells can directly inhibit B cells via IL-10 signaling in a model of food allergy [40].

B-cell intrinsic STAT6-dependent regulation of the IgE and GC response in mice

Antigen-activated B cells are required to integrate extrinsic signals from T cells and other sources to alter their gene expression profile and differentiate into GC B cells, undergo CSR and further develop into memory B cells and PCs. The transcription factor STAT6 plays a key role for these processes in type 2 immune responses.

STAT6 promotes CSR to IgE and IgG1 by direct binding to DNA elements in the germline ϵ and $\gamma 1$ promoters [41–43].

This process is enhanced by the poly ADP-ribosyl polymerase PARP-14 by release of inhibitory histone deacetylases (HDACs) from STAT6 binding sites [44, 45]. In addition, STAT6 induces expression of the transcription factor NFIL3 which is also required for CSR to IgE [46]. B cells can switch from IgM to IgE expression either directly or sequentially with an intermediate step of IgG1 expression. Sequential IgE CSR is required for production of affinity-matured IgE and for the memory IgE response [47, 48]. IgE⁺ GC B cells are very rare and this might be explained by IgE BCR-mediated signals that promote GC exit and differentiation to short-lived PCs independently of antigen binding [49, 50]. The IgE BCR was further shown to inhibit the formation of memory B cells and long-lived PCs [51].

In addition to promoting CSR to IgE and IgG1, B cell-intrinsic STAT6 is required for GC formation in type 2 immune responses to helminths or allergens but not for GC formation in response to viral infections [17]. STAT6 controls expression of well over 100 genes in B cells and the function of most STAT6-regulated genes in the context of GC formation and the B-cell fate during type 2 immune responses remains to be explored [52]. STAT6 promotes expression of MHC-II and CD86 on B cells, which could contribute to better interaction with antigen-specific T cells [42]. STAT6 further acts together with NF- κ B to induce expression of AID [53]. Caspase-6 is another STAT6-regulated gene which appears to be required for CSR to IgG1 and PCs differentiation [54]. Activated STAT6 also promotes the upregulation of the low-affinity IgE receptor CD23 on the surface of B cells and this may lead to uptake of IgE-bound antigens that can then be presented to CD4 T cells [42, 55, 56]. Functional characterization of further STAT6-regulated proteins will help to better understand the GC response in type 2 immunity.

In addition to STAT6, GC formation and IgG1 CSR is also promoted by IL-21-induced activation of STAT3 in B cells [20, 21]. However, STAT3 inhibits IgE CSR in a B cell-intrinsic manner and this effect can be overcome by strong CD40 signaling [21, 57] (Fig. 2). How exactly STAT3 inhibits IgE CSR and promotes IgG1 CSR remains to be investigated. Another transcription factor that inhibits IgE CSR is BCL-6 which shares several DNA recognition sites in the genome with STAT6 including binding sites in the germline ϵ promoter [58–60]. On the other hand, BCL-6 is required for GC formation [60]. The high expression level of BCL-6 in GC B cells could therefore explain the relatively low frequency of IgE⁺ GC B cells in various settings of type 2 immune responses.

PCs development and memory B-cell responses in type 2 immunity

PCs are the major population of antibody-producing cells. They are generated during each step of the immune response, and therefore, differ in their phenotype, half-life, affinity, and type of secreted antibody. Early PCs are derived from B cells that do not enter GCs or exit GCs at an early phase of the immune response and therefore acquired only few somatic mutations. They can provide first initial protection against pathogens and help to capture

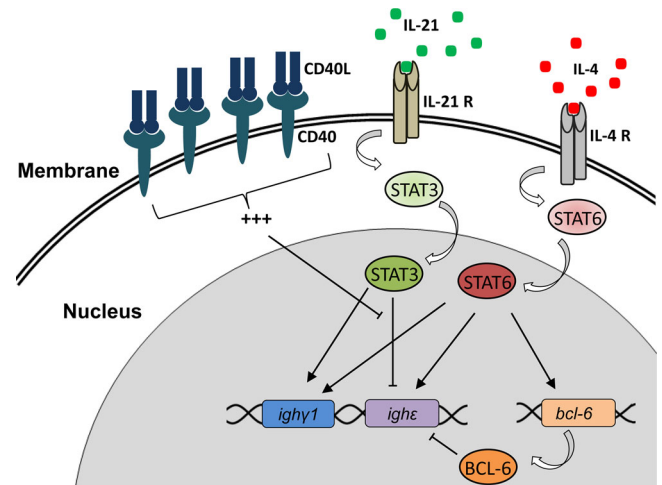


Figure 2. B-cell intrinsic regulation of IgG1 and IgE CSR in the humoral type 2 immune response. Signaling via the IL-4 receptor (IL-4 R) leads to activation of the transcription factor STAT6 which translocates into the nucleus thus activating expression of germline transcripts of IgE and IgG1, and transcription of BCL-6. BCL-6 in turn interferes with STAT6 binding to the IgE locus inhibiting transcription. Signaling via the IL-21 receptor (IL-21 R) leads to activation and translocation of STAT3 which in turn further promotes IgG1 CSR but inhibits IgE CSR. The STAT3 dependent inhibition of IgE can be overcome by strong signaling from CD40 activated by CD40L ligand (CD40L).

and therefore provide antigen for the GC reaction itself [5, 61]. In contrast, PCs produced during or at the end of the GC reaction are characterized by secretion of affinity matured and Ig class-switched antibodies.

Differentiation of B cells into PCs is strictly regulated by Tfh and Tfr cells. PC differentiation is promoted by interaction between ICOS and CD40L expressed on Tfh cells and ICOS-L and CD40 expressed on GC B cells [61]. A high BCR affinity would further improve this interaction thus favoring high affinity B cells to develop into PCs that leave the GC. Tfr cells on the other hand prevent the generation and GC exit of PCs that produce unspecific or autoreactive antibodies [38, 40, 62].

The half-life of IgE⁺ PCs is about 60 days in mice and four times shorter than the half-life of IgG1⁺ PCs [63]. However, chronic allergen exposure can cause accumulation of IgE⁺ PCs in the BM of both mice and humans [64]. The same IgE clonotypes can be detected in the peripheral blood of birch pollen allergic individuals in consecutive pollen seasons but not in off-season indicating that memory IgE persists long term at the clonal level [65]. IgE CSR may also occur within mucosal tissues as clonally related IgE⁺ and other isotypes have been recently identified in stomach and duodenum of human subjects affected by peanut allergy [66].

IgE-producing PCs express IgE BCRs on the cell surface (mIgE). This mIgE contains a so-called immunoglobulin tail tyrosine (ITT) motif in the cytoplasmic tail and a Tyr $\geq$ Phe mutation within this motif or deletion of the mIgE tail results in impaired memory IgE responses to helminths or allergen [67]. The ITT motif promotes surface expression of mIgE on PCs and due to the autonomous signaling capacity of mIgE may thereby promote

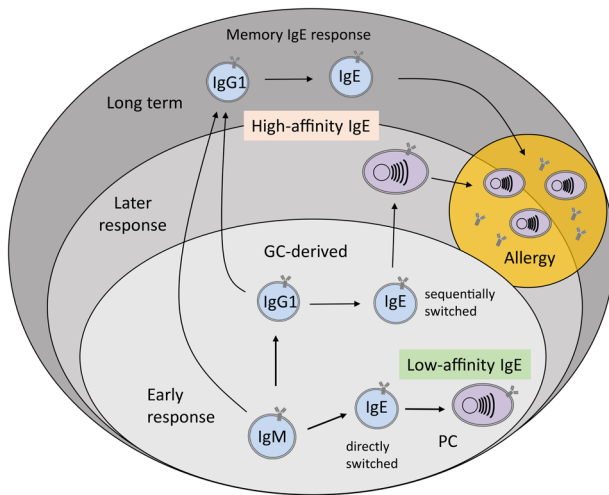


Figure 3. Progressive differentiation of IgE-producing plasma cells (PCs). PCs can be generated during early type 2 immune responses by direct IgM to IgE switching resulting in production of low-affinity IgE which does not elicit allergic reaction. Later immune responses generated sequentially switched, GC-dependent and high affinity IgE-producing PC. The cytoplasmic tail of the IgE BCR promotes the accumulation of IgE+ PC.

survival of IgE-producing PCs. This indicates that IgE memory persists either in the form of long-lived PCs that can be directly reactivated or in the form of IgG1+ memory B cells that immediately give rise to sequentially switched IgE+ PCs upon antigen encounter (Fig. 3). The memory IgE response seems indeed to be conserved at the level of IgG1+ memory B cells, at least in mice [48, 63, 68]. The second step of the sequential switch to IgE during the memory phase requires again IL-4 from T cells [48, 63]. Exchange of the extracellular part of IgG1 with IgE sequences abolished the memory IgE response indicating that either IgG1+ memory B cells receive extrinsic survival signals through the IgG1 BCR or that IgE+ B cells cannot further differentiate into memory B cells.

The mIgE might be a good target to specifically delete IgE-producing PCs. Indeed, an antibody against the M1 prime epitope in the extracellular part of mIgE targets IgE+ PCs for phagocytosis and thereby prevents further IgE production [69, 70]. In another study, a humanized anti-IgE antibody was described that neutralizes soluble IgE and marks mIgE-expressing cells for phagocytosis [71].

Conclusions

The humoral type 2 immune responses leading to GC formation and production of protective or anaphylactic IgE antibodies against pathogens and allergens are controlled by several B-cell extrinsic and intrinsic mechanisms. The T cell-derived cytokines IL-4 and IL-21 play a critical role for development of productive GCs but they appear to have opposite functions regarding the IgE response where IL-4 promotes and IL-21 inhibits CSR to IgE. The regulation of gene expression and IgE CSR by STAT6, STAT3, and

BCL-6 in primary B cells remains an important field of research to identify further mechanisms that control the humoral type 2 immune response in vivo.

B cells that directly switch from IgM to IgE do not undergo affinity maturation in GCs but rather differentiate to short-lived PCs and this process is driven by autonomous signaling of the mIgE. There is now good evidence that the memory IgE response is conserved at the level of IgG1+ memory B cells that sequentially switch to IgE and then differentiate to IgE+ PCs upon rechallenge. IgE+ PCs also express mIgE and the cytoplasmic tail of mIgE promotes their expansion by mechanisms that are not yet understood. The recent discovery of IL-13 producing Tfh cells required for production of anaphylactic IgE in response to allergens puts a new layer of complexity on the regulation of IgE production. Tfh cells are required for control of the GC response in general but also the IgE response in a house dust mite model. How these cells control Tfh cells and B cells inside GCs and whether they also act on emerging PCs requires further investigations.

The discovery of new regulatory mechanisms that control GC formation and IgE production during type 2 immune response could help to develop novel therapeutic strategies for treatment of allergic diseases.

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Abbreviations: AID: activation-induced cytidine deaminase · CSR: class switch recombination · ITT: immunoglobulin tail tyrosine · ILC2: innate lymphoid cells · PCs: plasma cells · Tfh: T-follicular helper · Tfr: follicular regulatory T cells

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