

REVIEW

The role of the T follicular helper cells in allergic disease

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T follicular helper (Tfh) cells were discovered just over a decade ago as germinal centre T cells that help B cells make antibodies. Included in this role is affinity maturation and isotype switching. It is here that their functions become less clear. Tfh cells principally produce IL-21 which inhibits class switching to IgE. Recent studies have questioned whether the germinal centre is the main site of IgE class switching or IgE affinity maturation. In this review, I will examine the evidence that these cells are responsible for regulating IgE class switching and the relationship between Tfh cells and T helper 2 (Th2) effector cells.

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INTRODUCTION

Common allergic diseases involve both humoral (IgE) and cell-mediated T helper 2 (Th2) mechanisms that drive inflammation. In addition, Th2 cells also regulate isotype switching to IgE. This is certainly true *in vitro* and the essential signals shown to be CD40L and IL-4 and to a lesser extent IL-13. At the beginning of the twenty-first century, several groups reported that a chemokine homing receptor CXCR5 was expressed on a unique population of T cells with specialized functions for switching B cells and affinity maturation. These cells were called T follicular helper (Tfh) cells and they were found in germinal centres (Figure 1).

TH1 AND TH2

The observation by Mosmann and colleagues that CD4 T cells could be subdivided according to their cytokine profile¹ and function² dramatically changed our understanding of what T cells did. Mosmann showed that Th1 CD4 T cells could enter peripheral tissues and recruit macrophages and neutrophils, while Th2 cells in the lung, for example, recruited eosinophils and drove mucus hypersecretion and airway hyper-responsiveness. What was not clear was how these cells could mediate both sets of functions. Expression of CCR7 and CD6L helps explain how central memory T cells are recruited to lymph nodes and CCR2 has been shown to facilitate effector memory T cells' entry to the lung, gut and other tissues. However, the distinction between CD4 T cells that help B cells and those that participate in cell mediated immunity is less clear. This has been a cause of considerable confusion to students who would try to separate these functions based on the Th1/Th2 paradigm.

WHAT ARE TFH CELLS

A series of papers at the beginning of the twenty-first century identified a subset of germinal centre CD4 T cells that differed from the T cells described above.^{3–5} These were first termed T follicular helper

(Tfh) cells by Chtanova *et al.*⁶ Germinal centre localized Tfh cells expressed the chemokine receptor CXCR5 as well as costimulatory molecules CD40L⁷ and ICOS.⁸ IL-21 is required for germinal centre formation for T-cell dependent antigens.⁹ However, Tfh CD4 T cells can secrete both IL-4 and IL-10¹⁰ as well as IL-21.⁶ Recently, Tfh cells have been shown to make IL-4 but not IL-13, while tissue Th2 cells made both IL-4 and IL-13.¹¹ Furthermore, Tfh cells that made IL-4 expressed low levels of GATA-3, whereas Th2 cells expressed high levels of GATA-3. Another transcription factor that may drive IL-4 in Th2 cells is conserved non-coding sequence 2.¹² conserved non-coding sequence 2-active cells have been found in germinal centres and expressed CXCR5 and Bcl6¹³ as well as IL-21 and IL-4. One transcription factor that directs Tfh differentiation is Bcl-6.¹⁴ Thus, Tfh and Th2 cells show clear differences, although it is unclear how these arise.

WHAT IS THE ALLERGIC IMMUNE RESPONSE?

Allergic disease is driven by both IgE and Th2 inflammatory processes that synergize. Why some organs are more or less affected is unclear, but there are tissue specific susceptibilities. Models in rats^{15–17} and subsequently in mice¹⁸ have shown that Th2 cells alone can drive many of the pathological processes seen in asthma including eosinophil infiltration, IL-4,¹⁹ IL-5²⁰ and IL-13 that causes mucus hypersecretion and airway hyper-responsiveness.²¹

HOW IS IGE CLASS SWITCHING CONTROLLED?

Immunoglobulin class switching by B cells is dependent on two signals. The first is delivered by specific cytokines that activate the promoter of the relevant immunoglobulin isotype²² and the second by CD40L expressed on the CD4 T cells.²³ IL-4 was shown²⁴ to switch B cells to IgE and subsequently interferon- γ was identified as the switch factor for IgG1 in humans²⁵ and IgG2a in the mouse.²⁶ To these was added transforming growth factor- β as the switch factor for IgA,²⁷ IL-10 a switch factor for human IgG4²⁸ and IL-21 a switch factor for

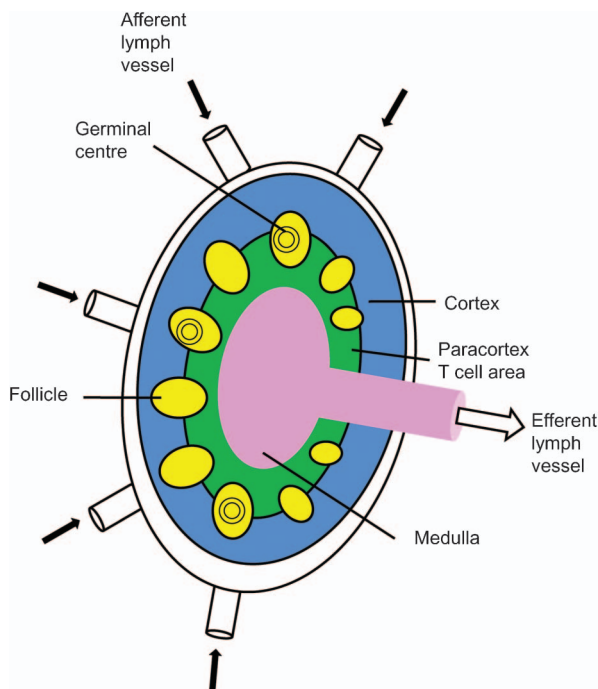


Figure 1 The conventional view of lymph node organization. Dendritic, T and B cells enter the lymph node through the afferent lymphatic vessels. Dendritic cells carrying antigen migrate to the paracortex where they may exchange antigen with interdigitating cells (resident dendritic cells). Naive T and B cells are recruited by antigen expressing dendritic cells and migrate to the germinal centres. The nature of the signals received from the dendritic cell as well as the affinity of the T cell for the antigen will determine the type of T helper response. The precise origin of Tfh cells as distinct from T helper is still unclear. Tfh, T follicular helper.

human IgG1 and IgG3.²⁹ Each of these cytokines is believed to be made by a different CD4 T cell subset.

ARE THERE TFH SUBSETS?

Since specific cytokines switch B cells to express different immunoglobulin isotypes and because these inhibit each other, are there Tfh subsets that correspond to different Th subsets—Th1, Th2, Th3, Th17 and so forth? Using an IL-21 reporter mouse, Luthje and colleagues³⁰ showed that IL-21 producing Tfh cells are multifunctional and can make IL-4 with or without IL-21, IL-21 with interferon- γ and IL-21 with IL-10. In the gut, Tsuji *et al.* reported that Tfh cells can arise from gut Foxp3⁺ cells.³¹ There are still uncertainties about the relationship between the development of Tfh and Th cells (Figure 2).

WHEN AND WHERE DOES CLASS SWITCHING OCCUR?

In response to repeated ovalbumin (OVA) aerosol, the highest number of OVA-specific IgE plasma cells was detected first in the lower respiratory tract (anterior and posterior mediastinal lymph nodes) and a smaller response in the upper respiratory tract (superficial cervical and internal jugular lymph nodes); indeed IgE mRNA was also detected in the lung and trachea by northern blot.³² In humans, IgG and IgA are produced locally as evidenced by the higher proportion of specific antibody to total immunoglobulin in nasal secretions.³³ Likewise, evidence for local IgE production can be found in tears.³⁴ Patients who are allergic to bee venom may remain sensitive decades after their last exposure. In patients with seasonal allergies, IgE antibodies continue to be produced between one season and the next when re-exposure to the allergen boosts their IgE and IgG response.³⁵

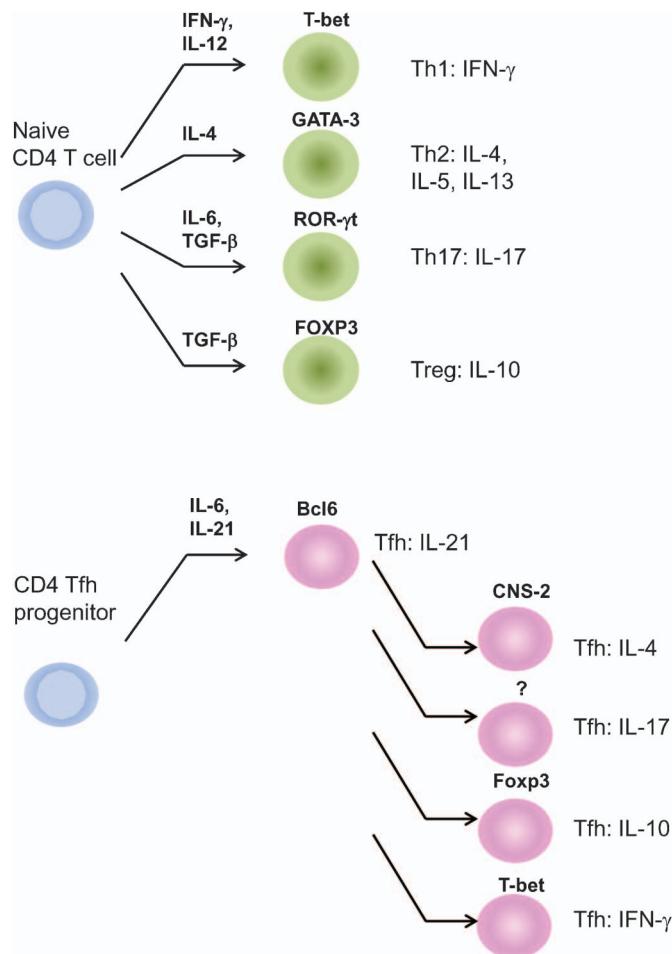


Figure 2 CD4 T-cell and possible Tfh cell subsets. Naive T cells differentiate into different subsets depending on the strength of the signal delivered by the interaction of the T cell receptor and peptide MHC and the cytokine microenvironment. While this has become quite clear for regular CD4 T-cell subsets, it is much less certain for Tfh cells. IFN, interferon; Tfh, T follicular helper; TGF, transforming growth factor.

HOW ARE TFH CELLS INVOLVED IN IGE CLASS SWITCHING?

IgE and other immunoglobulin isotypes are switched under the influence of T cell-derived cytokines and CD40L stimulation. Tfh cells most notably produce IL-21.^{30,36} IL-21 inhibits switching to IgE and IL-21R-deficient mice make high levels of IgE,^{37,38} while intranasal administration of recombinant IL-21 reduced the IgE response to ovalbumin as well as T-cell production of Th2 cytokines.³⁹ IL-21 inhibits germ line epsilon transcription in IL-4 stimulated B cells.⁴⁰ However, in humans, IL-21 enhanced IgE production from peripheral blood mononuclear cells cultured with IL-4 and stimulated with anti-CD40.⁴¹ IL-21 augmentation of IL-4-driven IgE switching in human B cells appears to be STAT3-dependent.⁴²

Using a novel system that included both T-cell receptor transgenic (DO11.10) and anti-influenza haemagglutinin B-cell receptor knock in mice, Erazo and colleagues⁴³ reported that although switching to IgE occurred in germinal centres, these cells contained the sterile IgE transcript, while IgE B cells found outside germinal centres predominantly made the mature transcript. They also showed that very few IgE-positive B cells were present in the germinal centre following immunization and proposed that few B cells switch directly to IgE in germinal centres, rather than IgG1 secreting cells that underwent

somatic hypermutation before becoming high-affinity IgE B cells once outside lymph nodes. More recently they have provided evidence that direct switching *via* IgE results in low-affinity IgE, while high-affinity IgE required sequential switching from IgG1 positive B cells.⁴⁴ This is supported by Wesemann *et al.*⁴⁵ who proposed that most mature B cell switching to IgE is *via* IgG1 and that direct IgM to IgE switching is a feature of immature B cells that may bypass hypermutation and selection in germinal centres. Recently, biocore has been used to monitor IgE affinity in *Cynomolgus* monkeys⁴⁶ and demonstrate IgE antibody affinity maturation. Additionally, the effect of IL-21 may involve the transcription factor, Bcl6, which rescues B cells from IL-21 induced cell death⁴⁷ and is expressed at low levels in plasma cells compared with germinal centre B cells, suggesting that switching to IgE may obey different rules at different stages of B-cell development.⁴⁸

An alternative view has been proposed by Talay and colleagues⁴⁹ who used green fluorescent protein linked to membrane form of IgE and found that most of the IgE detected in their model following *Nippostrongylus braziliensis* infection or immunization with TNP-OVA was directly from IgE positive germinal centre B cells. Subsequently, Yang *et al.*⁵⁰ have used another model in which the membrane form of IgE is linked to 'Venus', a yellow fluorescent protein with increased sensitivity.⁵¹ These authors also found that B cells that expressed IgE differentiated into both germinal centre B cells and IgE-positive plasma cells. IgE-switched B cells with the characteristics of, and located in, the germinal centre cells were identified. IgE memory B cells rather than IgG1 memory B cells appeared to be the major contributor to B-cell IgE memory. Mice, in which the gamma 1 switch is mutated, showed that switching to IgE could still occur and that serum *Nippostrongylus*-specific IgE levels were unaffected.⁵² Furthermore, removal of Sy1 increased the amount of OVA-specific IgE made by immunized mice.⁵³ *In vitro*, both sequential switching to IgE^{54,55} and direct IgM to IgE switching have been reported.⁵⁶ Thus, it is still unclear which mechanism is dominant and under what circumstances. A confounding factor may be B- and T-cell attenuator⁵⁷ that is highly expressed on CXCR5⁺ Tfh cells and inhibits their capacity to produce IL-21.⁵⁸

HOW DO HUMANS COMPARE WITH MICE?

Investigation into the development of allergy in humans is limited by access to the relevant tissues and almost exclusively relies on measurements of peripheral blood cells or soluble factors. Patients with atopic dermatitis do have circulating IgE secreting plasma cells.⁵⁹ Furthermore, we know that making an IgE response to foods such as egg is common in industrialized countries⁶⁰ and that these IgE antibodies give rise to symptoms. Like our laboratory mice, ovalbumin is not a typical allergen and continued exposure induces tolerance. Although IgE responses to egg take longer to decline in infants than in mice, the majority have little IgE antibody to egg by the time they are 2–3 years old and clinically most infants outgrow their egg allergy. The IgE response to inhalant allergens does not really start until 4–5 years of age and requires repeated low dose exposure and plenty of opportunity for the inflammation triggered by IgE bearing mast cells and its interaction with inflammatory Th2 cells. This is very difficult to recapitulate in animal models.

CONCLUSION

Considerable progress has been made to understand the regulation of the allergic immune response. We know that the two main drivers of disease are Th2 CD4 T cells and IgE attached to mast cells and basophils. We have a good understanding of how Th2 cells arise and the

factors that regulate their differentiation. What has proved more challenging is to understand how and where IgE responses take place. Similarly, there are still questions about the relationship between effector Th1 and Th2 cells and Tfh cells that have yet to be adequately resolved. The life history of IgE memory B cells and the location of IgE plasma cells in allergic patients require further study. We need to know what happens with real allergens such as grass pollen and dust mite. Nevertheless, we have an exciting range of tools at our disposal and it seems reasonable to expect that these questions will be resolved over the next decade.

- 1 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; **136**: 2348–2357.
- 2 Cher DJ, Mosmann TR. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by TH1 clones. *J Immunol* 1987; **138**: 3688–3694.
- 3 Walker LS, Gulbranson-Judge A, Flynn S, Brocker T, Raykundalia C, Goodall M *et al.* Compromised OX40 function in CD28-deficient mice is linked with failure to develop CXC chemokine receptor 5-positive CD4 cells and germinal centers. *J Exp Med* 1999; **190**: 1115–1122.
- 4 Schaerli P, Willmann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med* 2000; **192**: 1553–1562.
- 5 Kim CH, Rott LS, Clark-Lewis I, Campbell DJ, Wu L, Butcher EC. Subspecialization of CXCR5⁺ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5⁺ T cells. *J Exp Med* 2001; **193**: 1373–1381.
- 6 Chtanova T, Tangye SG, Newton R, Frank N, Hodge MR, Rolph MS *et al.* 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* 2004; **173**: 68–78.
- 7 Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M *et al.* Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med* 2000; **192**: 1545–1552.
- 8 Bossaller L, Burger J, Draeger R, Grimbacher B, Knöth R, Plebani A *et al.* ICOS deficiency is associated with a severe reduction of CXCR5⁺CD4 germinal center Th cells. *J Immunol* 2006; **177**: 4927–4932.
- 9 Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity* 2008; **29**: 127–137.
- 10 Butch AW, Chung GH, Hoffmann JW, Nahm MH. Cytokine expression by germinal center cells. *J Immunol* 1993; **150**: 39–47.
- 11 Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat Immunol* 2012; **13**: 58–66.
- 12 Tanaka S, Tsukada J, Suzuki W, Hayashi K, Tanigaki K, Tsuji M *et al.* The interleukin-4 enhancer CNS-2 is regulated by Notch signals and controls initial expression in NKT cells and memory-type CD4 T cells. *Immunity* 2006; **24**: 689–701.
- 13 Harada Y, Tanaka S, Motomura Y, Harada Y, Ohno S, Ohno S *et al.* The 3' enhancer CNS2 is a critical regulator of interleukin-4-mediated humoral immunity in follicular helper T cells. *Immunity* 2012; **36**: 188–200.
- 14 Yu D, Rao S, Tsai LM, Lee SK, He Y, Sutcliffe EL *et al.* The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* 2009; **31**: 457–468.
- 15 Watanabe A, Mishima H, Renzi PM, Xu LJ, Hamid Q, Martin JG. Transfer of allergic airway responses with antigen-primed CD4⁺ but not CD8⁺ T cells in Brown Norway rats. *J Clin Invest* 1995; **96**: 1303–1310.
- 16 Haczk A, Macary P, Haddad EB, Huang TJ, Kemeny DM, Moqbel R *et al.* Expression of Th-2 cytokines interleukin-4 and -5 and of Th-1 cytokine interferon-gamma in ovalbumin-exposed sensitized Brown-Norway rats. *Immunology* 1996; **88**: 247–251.
- 17 Haczk A, Macary P, Huang TJ, Tsukagoshi H, Barnes PJ, Kay AB *et al.* Adoptive transfer of allergen-specific CD4⁺ T cells induces airway inflammation and hyperresponsiveness in Brown-Norway rats. *Immunology* 1997; **91**: 176–185.
- 18 Kaminuma O, Mori A, Ogawa K, Nakata A, Kikkawa H, Naito K *et al.* Successful transfer of late phase eosinophil infiltration in the lung by infusion of helper T cell clones. *Am J Respir Cell Mol Biol* 1997; **16**: 448–454.
- 19 Cohn L, Homer RJ, Marinov A, Rankin J, Bottomly K. Induction of airway mucus production by T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. *J Exp Med* 1997; **186**: 1737–1747.
- 20 Hogan SP, Koskinen A, Matthaei KI, Young IG, Foster PS. Interleukin-5-producing CD4⁺ T cells play a pivotal role in aeroallergen-induced eosinophilia, bronchial hyperactivity, and lung damage in mice. *Am J Respir Crit Care Med* 1998; **157**: 210–218.
- 21 Mattes J, Yang M, Siqueira A, Clark K, MacKenzie J, McKenzie AN *et al.* IL-13 induces airways hyperactivity independently of the IL-4R alpha chain in the allergic lung. *J Immunol* 2001; **167**: 1683–1692.
- 22 Gauchat JF, Lebman DA, Coffman RL, Gascan H, de Vries JE. Structure and expression of germline epsilon transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J Exp Med* 1990; **172**: 463–473.

- 23 Armitage RJ, Fanslow WC, Strockbine L, Sato TA, Clifford KN, Macduff BM *et al.* Molecular and biological characterization of a murine ligand for CD40. *Nature* 1992; **357**: 80–82.
- 24 Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. *J Immunol* 1986; **136**: 949–954.
- 25 Kawano Y, Noma T, Yata J. Regulation of human IgG subclass production by cytokines. IFN-gamma and IL-6 act antagonistically in the induction of human IgG1 but additively in the induction of IgG2. *J Immunol* 1994; **153**: 4948–4958.
- 26 Snapper CM, Paul WE. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987; **236**: 944–947.
- 27 Kim PH, Kagnoff MF. Transforming growth factor beta 1 increases IgA isotype switching at the clonal level. *J Immunol* 1990; **145**: 3773–3778.
- 28 Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 1998; **160**: 3555–3561.
- 29 Pene J, Gauchat JF, Lecart S, Drouet E, Guglielmi P, Boulay V *et al.* Cutting edge: IL-21 is a switch factor for the production of IgG1 and IgG3 by human B cells. *J Immunol* 2004; **172**: 5154–5157.
- 30 Luthje K, Kallies A, Shimohakamada Y, Belz GT, Light A, Tarlinton DM *et al.* The development and fate of follicular helper T cells defined by an IL-21 reporter mouse. *Nat Immunol* 2012; **13**: 491–498.
- 31 Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T *et al.* Preferential generation of follicular B helper T cells from Foxp3⁺ T cells in gut Peyer's patches. *Science* 2009; **323**: 1488–1492.
- 32 McMenamin C, Girn B, Holt PG. The distribution of IgE plasma cells in lymphoid and non-lymphoid tissues of high-IgE responder rats: differential localization of antigen-specific and 'bystander' components of the IgE response to inhaled antigen. *Immunology* 1992; **77**: 592–596.
- 33 Platts-Mills TA. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 1979; **122**: 2218–2225.
- 34 Tuft SJ, Dart JK, Kemeny M. Limbal vernal keratoconjunctivitis: clinical characteristics and immunoglobulin E expression compared with palpebral vernal. *Eye* 1989; **3**: 420–427.
- 35 Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J Allergy Clin Immunol* 1982; **70**: 261–271.
- 36 Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L *et al.* Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* 2008; **29**: 138–149.
- 37 Ozaki K, Spolski R, Feng CG, Qi CF, Cheng J, Sher A *et al.* A critical role for IL-21 in regulating immunoglobulin production. *Science* 2002; **298**: 1630–1634.
- 38 Shang XZ, Ma KY, Radewonuk J, Li J, Song XY, Griswold DE *et al.* IgE isotype switch and IgE production are enhanced in IL-21-deficient but not IFN-gamma-deficient mice in a Th2-biased response. *Cell Immunol* 2006; **241**: 66–74.
- 39 Hiromura Y, Kishida T, Nakano H, Hama T, Imanishi J, Hisa Y *et al.* IL-21 administration into the nostril alleviates murine allergic rhinitis. *J Immunol* 2007; **179**: 7157–7165.
- 40 Suto A, Nakajima H, Hirose K, Suzuki K, Kagami S, Seto Y *et al.* Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. *Blood* 2002; **100**: 4565–4573.
- 41 Kobayashi S, Haruo N, Sugane K, Ochs HD, Agematsu K. Interleukin-21 stimulates B-cell immunoglobulin E synthesis in human beings concomitantly with activation-induced cytidine deaminase expression and differentiation into plasma cells. *Hum Immunol* 2009; **70**: 35–40.
- 42 Avery DT, Ma CS, Bryant VL, Santner-Nanan B, Nanan R, Wong M *et al.* STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. *Blood* 2008; **112**: 1784–1793.
- 43 Erazo A, Kutchukhidze N, Leung M, Christ AP, Urban JF Jr, Curotto de Lafaille MA *et al.* Unique maturation program of the IgE response *in vivo*. *Immunity* 2007; **26**: 191–203.
- 44 Xiong H, Dolpady J, Wabl M, Curotto de Lafaille MA, Lafaille JJ. Sequential class switching is required for the generation of high affinity IgE antibodies. *J Exp Med* 2012; **209**: 353–364.
- 45 Wesemann DR, Magee JM, Boboila C, Calado DP, Gallagher MP, Portuguese AJ *et al.* Immature B cells preferentially switch to IgE with increased direct Smu to Sepsilon recombination. *J Exp Med* 2011; **208**: 2733–2746.
- 46 Pol E, Karlsson R, Roos H, Jansson A, Xu B, Larsson A *et al.* Biosensor-based characterization of serum antibodies during development of an anti-IgE immunotherapeutic against allergy and asthma. *J Mol Recogn* 2007; **20**: 22–31.
- 47 Tsuruoka N, Arima M, Arguni E, Saito T, Kitayama D, Sakamoto A *et al.* Bcl6 is required for the IL-4-mediated rescue of the B cells from apoptosis induced by IL-21. *Immunol Lett* 2007; **110**: 145–151.
- 48 Kitayama D, Sakamoto A, Arima M, Hatano M, Miyazaki M, Tokuhisa T. A role for Bcl6 in sequential class switch recombination to IgE in B cells stimulated with IL-4 and IL-21. *Mol Immunol* 2008; **45**: 1337–1345.
- 49 Talay O, Yan D, Brightbill HD, Straney EE, Zhou M, Ladi E *et al.* IgE⁺ memory B cells and plasma cells generated through a germinal-center pathway. *Nat Immunol* 2012; **13**: 396–404.
- 50 Yang Z, Sullivan BM, Allen CD. Fluorescent *in vivo* detection reveals that IgE⁺ B cells are restrained by an intrinsic cell fate predisposition. *Immunity* 2012; **36**: 857–872.
- 51 Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A. A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nat Biotechnol* 2002; **20**: 87–90.
- 52 Jung S, Siebenkotten G, Radbruch A. Frequency of immunoglobulin E class switching is autonomously determined and independent of prior switching to other classes. *J Exp Med* 1994; **179**: 2023–2026.
- 53 Misaghi S, Garris CS, Sun Y, Nguyen A, Zhang J, Sebrell A *et al.* Increased targeting of donor switch region and IgE in Sgamma1-deficient B cells. *J Immunol* 2010; **185**: 166–173.
- 54 Zhang K, Mills FC, Saxon A. 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* 1994; **152**: 3427–3435.
- 55 Baskin B, Islam KB, Evengard B, Erntestam L, Smith CI. Direct and sequential switching from mu to epsilon in patients with *Schistosoma mansoni* infection and atopic dermatitis. *Eur J Immunol* 1997; **27**: 130–135.
- 56 Cameron L, Gounni AS, Frenkiel S, Lavigne F, Vercelli D, Hamid Q. S epsilon S mu and S epsilon S gamma switch circles in human nasal mucosa following *ex vivo* allergen challenge: evidence for direct as well as sequential class switch recombination. *J Immunol* 2003; **171**: 3816–3822.
- 57 Watanabe N, Gavrieli M, Sedy JR, Yang J, Fallarino F, Loftin SK *et al.* BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat Immunol* 2003; **4**: 670–679.
- 58 Kashiwakuma D, Suto A, Hiramatsu Y, Ikeda K, Takatori H, Suzuki K *et al.* B and T lymphocyte attenuator suppresses IL-21 production from follicular Th cells and subsequent humoral immune responses. *J Immunol* 2010; **185**: 2730–2736.
- 59 Dhanjal MK, Towler AE, Tuft S, Hetzel C, Richards D, Kemeny DM. The detection of IgE-secreting cells in the peripheral blood of patients with atopic dermatitis. *J Allergy Clin Immunol* 1992; **89**: 895–904.
- 60 Ruiz RG, Kemeny DM, Price JF. Higher risk of infantile atopic dermatitis from maternal atopy than from paternal atopy. *Clin Exp Allergy* 1992; **22**: 762–766.