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MRS. MUBECCEL AKDIS (Orcid ID : 0000-0003-0554-9943)

DR. WILLEM VAN DE VEEN (Orcid ID : 0000-0001-9951-6688)

Article type : Review

Biology and dynamics of B cells in the context of IgE-mediated food allergy

Pattraporn Satitsuksanoa[1], Monique Daanje[1], Mübeccel Akdis[1], Scott D. Boyd[2,3] Willem van de Veen[1,4]

[1]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland.

[2]Sean N. Parker Center for Allergy and Asthma Research, Stanford University School of Medicine, Stanford, CA 94305, USA.

[3]Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA.

[4]Christine Kühne-Center for Allergy Research and Education (CK-CARE), Davos, Switzerland.

Correspondence:

Willem van de Veen, PhD, Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Herman-Burchard-Strasse 9, CH-7265 Davos-Wolfgang, Switzerland
Tel.+41(0)814100846

Email: willem.vandeveen@siaf.uzh.ch

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ALL.14684

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Short title

B cells in IgE-mediated food allergy

Keywords

Antibodies

B cells

Food allergy

IgE

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Abbreviations

AD	Atopic Dermatitis
BCR	B cell receptor
Breg	Regulatory B cell
CD40L	CD40 ligand
CSR	Class switch recombination
D	Diversity
DC	dendritic cell
FcεRI	Fc epsilon RI receptor
GC	germinal center
IgE-FAP	IgE-facilitated antigen presentation
IGH	immunoglobulin heavy chain locus
MHC	major histocompatibility complex
OIT	oral immunotherapy
IL	interleukin
J	Joining
TGF-β	Transforming growth factor beta
Th	T helper
TFH	follicular T helper
Treg	regulatory T cells
TSLP	thymic stromal lymphopoietin
V	Variable

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Abstract

An increasing number of people suffer from IgE-mediated food allergies. The immunological mechanisms that cause IgE-mediated food allergy have been extensively studied. B cells play a key role in the development of IgE-mediated food allergies through the production of allergen-specific antibodies. While this particular function of B cells has been known for many years, we still do not fully understand the mechanisms that regulate the induction and maintenance of allergen-specific IgE production. It is still not fully understood where in the body IgE class switch recombination of food allergen-specific B cells occurs, and what processes are involved in the immunological memory of allergen-specific IgE responses. B cells can also contribute to the regulation of allergen-specific immune responses through other mechanisms such as antigen presentation and cytokine production. Recent technological advances have enabled highly detailed analysis of small subsets of B cells down to the single cell level. In this review, we provide an overview of the current knowledge on the biology of B cells in relation to IgE-mediated food allergies.

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Introduction

The prevalence of atopic diseases has been steadily increasing over the last decades. Food allergies are caused by adverse immune responses to food proteins and differ from food intolerance in that food intolerance is typically not caused by dysregulated immune responses but rather result from other mechanisms such as enzyme deficiencies.[1] Food allergies are atopic disorders that can be broadly classified into IgE-mediated, non-IgE-dependent or a combination of both.[2]

IgE-mediated food allergy is defined as an adverse immune response to food proteins which involves the production of allergen-specific IgE antibodies.[3] Clinical signs of food allergy are observed after ingestion of the allergen and include gastrointestinal disorders, airway inflammation, urticaria, and, in severe cases, systemic anaphylaxis.[2] Food allergy is common in Europe with a prevalence of up to 10% in the pediatric population[4] and 1-3% in the adult population.[5] Furthermore, there is evidence that food allergies are increasing in prevalence.[5,6] The major food allergens in developed countries are peanut, tree nuts, fish, shellfish, sesame, milk, egg and soy.[4,7,8] Particularly peanut and tree nuts allergies frequently persist for a lifetime[3,5] and anaphylactic reactions upon allergen exposure can occur at any age. [3]Due to their high prevalence, food allergies are responsible for substantial health care costs.[9] Currently, the approach to manage food allergy is focused on avoidance of culprit foods while allergen-specific oral immunotherapy (OIT) is becoming increasingly recognized as a treatment option.[10]

B cells play a pivotal role in IgE-mediated food allergies, as a result of their unique ability to produce allergen-specific IgE antibodies that sensitize mast cells and basophils by binding to their high affinity IgE receptors (FcεRI).[11] Subsequent allergen crosslinking of FcεRI-bound IgE on mast cells and basophils initiates the release of pro-inflammatory mediators resulting in a type I hypersensitivity reaction. [12] Immune regulatory functions of B cells, particularly regulatory B cells (Breg), have also been described.[1314] Serological mechanisms of immune regulation by B cells include the prominent increase of IgG4 antibodies in serum observed in patients gaining immune tolerance, as seen during oral immunotherapy against peanut allergy.[15] Clearly, B cells are fundamental in both developing food allergies and gaining tolerance (**Figure 1**).

This review focuses on the role of B cells in IgE-mediated food allergy and allergen-tolerance, elaborating on the mechanisms underlying IgE production, the immunological

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memory of the IgE response, the role of B cells in the gastrointestinal tract, and cellular responses of B cells.

IgE induction in food allergy

IgE-mediated food allergies are characterized by a T helper 2 (Th2) skewed immune response towards otherwise innocuous food proteins.[16] Sensitization to food allergens can occur via allergen exposure to gut epithelial cells, but also via allergen exposure to damaged skin. This might explain the high prevalence of food allergies among patients with atopic dermatitis (AD).[17,18] AD appears to be associated with higher levels of IgE somatic hypermutation (SHM). Indeed, recent data from longitudinal analysis of the B cell repertoires have demonstrated that infants with AD show increased IgE SHM, even within the first two years of life.[19] Because more mutated antibody genes often correlate with increased affinity of the antibody, elevated IgE SHM may be an important contributing factor to the development of allergic disease.[20]

Key epithelial cytokines leading to the allergic response include interleukin (IL-)25, IL-33 and thymic stromal lymphopoietin (TSLP), which induce a shift towards Th2 cell differentiation by activating type 2 innate lymphoid cells, basophils, and dendritic cells (DCs).[21,22] Upon exposure to the antigen, DCs engulf the antigen and migrate to the lymph node to present an antigen-derived peptide to naive cognate T-cells, which become activated and undergo clonal expansion[22] Afterwards, IgE class switch recombination, plasma cell differentiation and allergen-specific IgE production may occur locally in the GI tissues including stomach and duodenum as well as in secondary or tertiary lymphoid organs (**Figure 2**).

Human intestinal epithelial cells can directly take up allergen-IgE complexes via the low-affinity IgE receptor CD23.[23] CD23 is a type II transmembrane glycoprotein with a carboxy terminal C-type lectin head that binds to IgE.[24] Human CD23 exists in two different isoforms: CD23a and CD23b. These two isoforms display distinctive functions, particularly, CD23a has been shown to act as a bidirectional transporter of IgE across the intestinal epithelial barrier.[25] Interestingly, food allergens that bind to CD23 as allergen-IgE complexes are protected from lysosomal degradation in gut epithelium.[26] As a result, these food antigenic complexes are conserved during the transcytosis and later captured

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by gut DCs or activated mast cells. For this reason, allergen-IgE complexes with CD23 mainly engage in food-induced pathophysiology of the gastrointestinal tract.

B cells carrying a specific B cell receptor (BCR) for the allergen can capture allergens directly or indirectly through follicular DCs, and present allergen-derived peptides to cognate CD4+ T cells. Antigen-stimulated B cells can be activated upon interaction between CD40 on B cells and CD40 ligand (CD40L) on activated CD4+ T cells, together with the secretion of type 2 cytokines such as IL-4, IL-5, IL-13 and IL-9.[12] IL-9 is often produced along with IL-4 by Th2 cells and the major source of IL-9 is mucosal mast cells in intestine.[27]

Although TH2 cells have long been considered the main cell type responsible for the induction of class switching recombinant (CSR) to IgE, more recent studies indicate that this process is largely dependent on T follicular helper (TFH) cells.[28] A distinct subset of TFH cells, has recently been reported to drive the production of high-affinity IgE antibodies to respiratory and food allergens. These cells termed TFH13 cells are capable of producing high levels of IL-4, IL-13 and IL-5 and relatively modest levels of the prototypical TFH cytokine IL-21.[29] Activated B cells stimulated by Th2 cytokines can undergo CSR to IgE and can subsequently differentiate into IgE+ plasma cells.[12] It should be noted that the anatomical sites of CSR, either at the margin of GCs, or within GCs, or in extrafollicular sites, are still a topic of active investigation, particularly for IgE. [30] There may also be significant differences between humans and mice in IgE+ B cell differentiation sites.

IgE class swichting recombinant

A combination of CSR and affinity maturation is required for the generation of allergen specific high affinity IgE antibodies. CSR is a complex process that is tightly regulated and can result in the expression of an IgG1, IgG2, IgG3 IgG4, IgE, IgA1 or IgA2 heavy chain. [31] The immunoglobulin heavy chain locus (IGH) contains multiple constant (C) regions, which encode the non-variable part of the different immunoglobulin heavy chain isotypes. These C regions are located downstream of the variable heavy (V_H), diversity (D), and joining heavy (J_H) ($V_H(D)J_H$) cassette. Each C_H H gene has its own promoter, an intervening (I) exon and a switch (S) region directly upstream of the coding exons (**Figure 3A**).[32] In naïve B cells, the $V_H(D)J_H$ cassette is linked to the constant

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region of the μ chain (Cμ). Consequently, naïve B cells express surface IgM and, as a result of alternative splicing, IgD as their BCR. During CSR, double strand breaks are generated at two different S regions within the IgH locus, followed by deletion of the intervening DNA ligation of the remaining S regions (**Figure 3A**). In allergy, CSR takes place from IgM to IgE, either directly or sequentially. In humans, high-affinity IgE antibodies, particularly important in allergic diseases, are thought to result from sequential switching, most commonly via IgG1 and other IgG isotypes [33-36] but also from IgA1 based on evidence from gastrointestinal tissues. [37] **(Figure 3A, B)**

Although CSR is typically considered to happen in or near GCs in secondary lymphoid tissues, there is evidence that IgE CSR can occur at mucosal sites that lack organized GCs, such as the nasal mucosa in patients with respiratory allergies. [38-40] Recent evidence indicates that the mucosal tissues of the gastrointestinal tract in humans are also a site of local CSR and the differentiation of IgE+ plasma cells, even in tissues such as the stomach, which do not contain large organized secondary lymphoid structures such as Peyer's patches. Large numbers of IgE+ B lineage cells, including cells with a plasma cell phenotype, were detected in stomach and duodenum biopsies of peanut allergic patients but were largely absent in non-allergic controls. Stomach and duodenum IgE+ clone counts correlated with allergen-specific serum IgE concentrations, suggesting that gastrointestinal tissues may function as a reservoir for allergen-specific IgE+ plasma cells in peanut allergic patients. Non-IgE members of clonal lineages that contained multiple isotypes besides IgE were most frequently identified in the same tissue biopsy that also contained the IgE member of that clone. [37]The localized presence of IgE-expressing B-lineage cells together with other B cells from the same clones expressing other isotypes in the same tissue biopsies, as well as the greater sharing of clones between anatomically proximal tissues, are consistent with local class switching and limited migration of clone members in mucosal tissues.

Affinity maturation of IgE antibodies

Affinity maturation for most antibodies is thought to be the result of multiple rounds of somatic hypermutation in the GCs present in secondary lymphoid tissues. GCs consist of a dark zone that comprises proliferating B cells, and a light zone, in addition

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comprising follicular dendritic cells and TFH cells.[41] During affinity maturation, B cells migrate within GCs from the dark zone to the light zone and back. In the dark zone, B cells expand and generate random mutations in the VDJ-region of the antibody gene locus, resulting in B cells with a wide variety of affinities for the antigen. [42] After arriving in the light zone, B cells are in competition to take up antigen presented by follicular dendritic cells.[43] B cells with high affinity B cell receptors can take up the antigen most efficiently and present more antigen-derived peptides on their major histocompatibility complex (MHC) class II molecules, hence receiving most help from TFH cells.[42] As such, the high-affinity B cells are positively selected and subjected to another round of somatic hypermutation and expansion.[41] This process may account for the development of allergen-specific high-affinity IgE molecules in allergy but it is still an open question whether B cells that have already switched to IgE production take part in the GC reaction, or whether most or all of the affinity maturation takes place in non-IgE-expressing B cells that later switch to IgE. The latter possibility could account for the highly mutated and apparently affinity-matured IgE-expressing B cells seen in mucosal sites in humans.[37] Several studies using mouse models suggest that the affinity maturation of the IgE antibody response occurs in precursor IgG1 cells that sequentially switched to IgE. Mice that were deficient for class switching to IgG1 were able to produce low affinity IgE antibodies but could not produce high affinity IgE antibodies.[34] Thus putative sequentially switched IgE antibodies in mice generally showed higher levels of mutations in the V, D, and J germline regions, suggesting that the IgG1 intermediate was subject to SHM before switching the heavy chain to IgE. This is also supported by the finding that allergen-specific IgG1+ B cells preceded the development of IgE responses in a murine model of epicutaneous sensitization to food allergens.[44] Analysis of the IgH repertoire in allergic as well as non-allergic individuals also indicated that sequential CSR of mutated IgG1-switched B cells is the main source of mutated IgE in humans. Indications for sequential CSR to IgE through other switched isotypes were less frequent, while direct CSR to IgE from IgM or IgD was the least common route of IgE generation.[45]

Immunological memory and plasma cell IgE responses in food allergy

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Allergen-specific IgE antibodies can be detected in serum for extended periods of time even after prolonged periods without allergen exposure. This was clearly demonstrated in a cohort of patients that were treated for filarial infections and were not re-infected afterwards. In these patients, helminth antigen-specific IgE antibodies could be detected several years after clearance of the infection.[46] Similarly, data from patients undergoing bone marrow transplantation have shown that persistent allergies to foods or aeroallergens can be conferred from donor to recipient, presumably via transfer of memory B cells, or bone marrow plasma cells, or both.[47,48]

These data are consistent with IgE+ plasma cells as a long-lasting source of specific IgE antibodies independent of antigen exposure. However, there are also data indicating that ongoing or periodic memory B cell activation and differentiation to plasma cells contributes to the circulating allergen-specific IgE. Seasonal increases of pollen-specific IgE antibody titers suggest that short-lived IgE-producing plasma cells develop during the pollen season and temporarily increase allergen-specific IgE production.[49,50] The same seems to apply to food allergen-specific IgE, as the levels of food allergen-specific IgE was reduced in most, but not all hen's egg and cow's milk sensitized patients after a three months elimination diet.[51] In a mouse model of peanut allergy, allergen-specific IgE levels were not sustained over time in absence of continued allergen exposure, suggesting that, in this model, the IgE memory is not contained in the long-lived plasma cell compartment. Maintenance of clinically relevant allergen-specific IgE titers required B cell reactivation in response to allergen-exposure.[52]

Whether or not there exist IgE+ memory B cells in humans and mice has been a topic of debate, with some groups finding evidence of non-plasma cell IgE+ B cells [53-56] and data from mice favoring an IgG memory cell population as the main source of IgE+ plasma cells.[57,58] However, it must be noted that the method for detection of IgE-switched B cells through surface staining using an anti-IgE antibody followed by flow cytometry analysis may result in a high rate of false-positive events. Sorting of apparently IgE+ B cell by flow cytometry yielded a predominance of B cells that were found to express other isotypes when analyzed by single cell sequencing..[55,59] On the other hand, elevated numbers of IgE+ B cells and plasmablasts were observed in pediatric patients with IgE-mediated food allergies, indicating that there may be a population of circulating memory and plasma cells that are IgE-switched present in food allergic patients.[53] A recent study

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reported that chronic intranasal house dust mite administration (3x per week for 15 weeks) in a mouse model resulted in generation of long-lived IgE+ plasma cells in the bone marrow that were primarily derived from sequential CSR of IgG1, while short term exposure (3x per week for 4 weeks) resulted in accumulation of IgE+ B cells and plasma cells in the spleen. Moreover, IgE produced in chronically exposed animals was capable of inducing anaphylaxis, while short term exposed mice did not produce anaphylactogenic IgE.[60]

There is strong evidence that long-lived plasma cells do exist in primates and can contribute to durable serum titers even if memory B cells are depleted. [61] But are there any IgE+ plasma cells among this long-lived population? Although IgE+ plasma cells have been detected in bone marrow of allergic patients [60] as well as STAT3 hyper IgE patients [62], the longevity of these cells remains hard to ascertain. Experiments intended to deplete membrane-expressing IgE+ cells, or to prevent CSR to IgE by blocking IL-4 or IL-13 signaling, only partially suppressed serum IgE titers, which could indicate the presence of long-lived IgE+ plasma cells, but do not provide direct evidence for the existence of these cells. [63-65]

Protective antibody responses in food allergy

Besides their involvement in generating allergen-specific IgE antibodies that contribute to the pathogenesis of food allergy, B cells can also produce allergen-specific antibodies of other isotypes that may rather protect against food allergies. In mice, mucosal IgA was found to inhibit the uptake of antigens by epithelium and may protect against food allergy. [66] Other mouse studies suggest that allergen-specific IgG antibodies may offer protection against allergic reactions by means of allergen interception, as well as by signaling through the inhibitory IgG receptor FcγRIIb on mast cells and basophils. [67] Allergen-specific IgG antibodies can also mediate feedback inhibition on allergen-specific IgE production by B cells through simultaneous engagement of FcγRIIb and BCR leading to a reduction of antibody production by B cells. [68,69] In line with this protective response, administration of allergen-specific IgG antibodies during experimental sensitization to food allergens in a mouse model could prevent development of allergic responses and also enhanced tolerance induction in a desensitization model. [70] Santos *et al.* demonstrated that, while sera obtained from peanut sensitized but tolerant children did

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not cause peanut-induced mast cell activation, depletion of IgG4 from these sera of children resulted in peanut-induced mast cell activation. This suggests that IgG4 blocking antibodies may contribute to absence of clinical reactivity in peanut sensitized individuals. [71]

In particular allergen-specific antibodies of the IgG4 isotype have been associated with immune tolerance to allergens, as it has several non-inflammatory properties. IgG4 can form bispecific functionally monovalent antibodies through a process called Fab arm exchange. [72] These bi-specific IgG4 antibodies are impaired in their capacity to form immune complexes and may interfere with immune complex formation by other allergen-specific antibody isotypes. IgG4 antibodies also have a disrupted C1q binding site, leading to their inability to fix complement. Increases in serum allergen-specific IgG4 have been observed in response to allergen immunotherapy (AIT) and have also been reported in healthy subjects exposed to relatively high doses of allergens, such as non-allergic beekeepers and cat owners. [36,73]

While IgE and IgG4 have long been recognized as key players in the regulation of allergic responses[36], it was recently reported that IgD may have both enhancing and protective roles in IgE-mediated allergic responses.[74] A small subset of human B cells expresses IgD in absence of IgM. This has been attributed to transcriptional inactivation of the IgM locus and not to classical CSR.[75] IgD has a short serum half-life and constitutes less than 0.5% of the total serum immunoglobulins. It was demonstrated that certain antigens including cow's milk allergens and bee venom allergens can induce specific IgD production by B cells. Secreted IgD can bind to basophils through galectin-9, which interacts with CD44, leading to production of type 2 cytokines by basophils and promotion of Th2-mediated responses.[74] It was also found that IgD-activated mast cells can faciliate IgE production in chronic rhinosinusitis patients with nasal polyps, supporting this potential enhancing effect of IgD on allergic inflammation.[76] On the other hand, IgD may function as a protective mediator against type I hypersensitivity reactions as it was found to constrain IgE-mediated basophil and mast cell degranulation.[74] Interestingly serum IgD antibodies against milk-derived allergens alpha s1-casein, beta-lactoglobulin were increased in cow's milk allergic children after oral immunotherapy.[74] Similarly, in a cohort of HDM AIT-treated patients, allergen-specific IgD was significantly increased after 2 years of treatment.[77] Although the exact contribution of allergen-specific IgD antibodies

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in the context of IgE-mediated allergic inflammation remains incompletely understood, the currently available data suggests that IgD contributes to allergic sensitization through enhancing IgE production. On the other hand, IgD may also play a role in the induction of allergen tolerance by suppressing IgE-mediated basophil (and potentially also mast cell) degranulation.[76]

B cell-mediated antigen presentation

While it is widely recognized that B cells can function as antigen presenting cells [78], it is not fully understood to what extent antigen presentation by B cells contributes to IgE-mediated food allergy and/or induction of tolerance to food allergens. Antigen-specific B cells are highly efficient at capturing antigens and presenting antigen-derived peptides to CD4+ T cells. In addition, B cells can capture and present antigens through a process called IgE-facilitated antigen presentation (IgE-FAP). B cells that express the low affinity IgE receptor CD23 on their surface membrane can bind immune complexes consisting of allergen-specific IgE antibodies and allergens. This results in cross-linking of CD23, internalization of the immune complexes by means of receptor-mediated endocytosis and presentation of allergen peptides on MHCII molecules. [79] This process enhances the efficiency of allergen uptake and presentation by B cells that have a BCR that is not specific to the allergen. IgE-FAP was found to facilitate higher proliferative responses to peanut allergens in peanut allergic patients. [80] It was also shown that the presence of IgG in allergen-antibody complexes results in binding to B cells via complement receptor type 2 (CD21) in addition to CD23. [81] This indicates that the ratio of specific IgE to IgG has an effect on how these allergen-antibody complexes can bind to B cells, and might affect antigen processing and presentation as well as downstream T cell responses. Allergen-specific IgG4 may interfere with this process because of its functional monovalency resulting from Fab-arm exchange, which leads to the formation of bi-specific IgG4 antibodies. [72] As a consequence IgG4 antibodies are impaired in their capacity to form immune complexes and may counteract immune complex formation by other antibodies.

Regulatory B cells

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Induction and maintenance of immune tolerance to food antigens is crucial for restoring a healthy immune response in food-allergic patients.[2] B cells, which can differentiate into antibody-secreting plasma cells, enhance immune responses by producing antigen-specific antibodies and by providing help to induce optimal CD4+ T cell activation. [82] On the other hand, immunosuppressive Breg cells modulate immune responses by suppressing effector T cells through the production of suppressor cytokines such as IL-10, TGF-β, and IL-35.[83,84] They can suppress inflammatory responses in autoimmune diseases, cancer, transplantation, infections and allergies.[84] IL-10 plays a pivotal role in the induction of immune tolerance in allergic inflammation.[85] It promotes IgG4 production and suppresses IgE production. [86,87]

. Inducible IL-10 secreting B regulatory (Br1) cells in beekeepers play a major role in contributing to peripheral allergen tolerance and upregulated production of IgG4 in bee venom allergic patients after AIT. IL-10-overexpressing B cells suppress the maturation of dendritic cells and, T effector cell proliferation, and IgE production. Moreover, IL-10-overexpressing B cells secreted the anti-inflammatory IL-1 receptor antagonist and vascular endothelial growth factor and showed lower production of pro-inflammatory cytokines.[1388]

While Breg cells have been studied in the context of several allergic conditions [89], the role of cellular B cell responses in relation to food allergy remains elusive. Several mouse studies indicate a protective role for Breg cells in the context of food allergies. In a murine model of food allergy using cow's milk-derived casein, a subset of IL-10-producing CD5+ Breg cells in mesenteric lymph nodes, but not in spleen or peritoneal cavity, suppressed IgE-mediated anaphylaxis following challenge with casein. This protective effect was dependent on Foxp3+ regulatory T cells (Tregs), indicating that mesenteric IL-10-producing Breg cells suppress food allergy via induction of Tregs.[90]

The induction of cellular Breg cell responses in mucosal sites could be highly relevant for protection against food allergies. It was found that human type 3 innate lymphoid cells support the maintenance of mucosal tolerance. Human CD40L+ type 3 innate lymphoid cells provide innate B cell help and induce Breg cells differentiation, which takes place in palatine tonsils *in vivo*.[91] The microbiome also plays a key role in the induction of Breg cells, as it was found that antibiotic treatment and sterile housing condition led to a reduction in Breg cell differentiation and IL-10 production compared to

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conventionally housed mice.[92] In this context it is important to note that the gut microbiome is involved in the regulation of many aspects of the immune response to food allergens. [93] The role of Breg cells and the processes that drive their differentiation and activation at mucosal sites in relation to food allergy and tolerance to food allergens still remains incompletely understood and warrants further studies.

Microbiome in food allergy

The gut microbiome plays an important role in the protection against food allergy by balancing and inducing tolerogenic immune function.[94] The imbalance of gut microbiota composition can cause allergic inflammation and is called "dysbiosis". Evidence from human and mouse studies implies that dysbiosis is strongly associated with the pathogenesis of food allergy.[95-97] Rosser *et al.* demonstrated that gut microbiota stimulate the differentiation of Breg cell in the spleen and mesenteric lymph node from antibiotic-treated mice compared to wild-type mice.[92] In an antigen-induced arthritis murine model, the immunosuppressive CD19+CD21hiCD23hiCD24hiCD1dhi Breg cell population was significantly reduced in antibiotic-treated mice. Subsequently the induction of arthritis, mice produced IL-1b and IL-6 cytokines, which directly regulate Breg cell differentiation and IL-10 production. Overall, their results indicate a crucial role for commensal microbiota in the induction of Breg cells . In addition, Mishima *et al.* demonstrated that commensal bacteria activated intestinal IL-10–producing B cells through TLR2, MyD88, and PI3K pathways.[98] They found that microbiota from specific pathogen–free mice activates IL-10–producing colon-specific B cells and Treg cells in germ-free mice. These IL-10–producing B cells then reduced colonic T cell stimulation and preserved mucosal homeostasis. Thus, the intestinal microbiome seems to be an important factor that acts on Breg cells to control intestinal inflammation. However, further studies are warranted to better understand the interaction between the microbiome and B cell responses in relation to IgE-mediated food allergies.

Allergen-specific B cells in food allergy and immunotherapy

Although accurate quantification of allergen-specific B cells is difficult as a result of their low frequency in circulation, several groups have established methods to detect and quantify allergen-specific B cells. Estimates of circulating allergen-specific B cells range from <0.03% of class-switched memory B cells in peanut allergic individuals [99,100] to

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<0.3% of class-switched memory B cells in bee venom allergic patients [101] and house-dust mite allergic patients. [102]

The few published studies aimed at characterization of food allergen-specific B cells have focused on peanut allergens. The frequencies of circulating B cells specific for the peanut allergens Ara h 1- and Ara h 2 were found to increase in response to OIT. [99,100] Interestingly, one study reported that the increase of Ara h 2-binding memory B cells peaked after 7 weeks of peanut OIT and then started to decline to levels that were lower than before OIT. [100] Besides quantitative analysis of peanut allergen-specific B cell frequencies in circulation, there is no human data available on the cellular B cell responses in food allergy and food immunotherapy. The advent of highly sensitive single cell transcriptomics techniques that allow capture of immunoglobulin heavy and light chain V(D)J sequences in conjunction with the full transcriptome of individual cells will allow highly detailed analysis of antigen-specific B cells. [103] Although this type of analysis has already been used to identify and characterize allergen-specific IgE-switched B cells and/or plasmablasts in peanut allergic patients [55], studies in which allergen-specific B cell responses are studied during the course of OIT have not been published.

Discussion

Our understanding of the role of B cells in IgE-mediated food allergy has significantly improved in recent years. We understand now that B cells play a multifaceted role in the regulation of IgE-mediated food allergies. They contribute to the pathogenesis of these allergies through production of high affinity IgE antibodies which cause the type I hypersensitivity reactions that are central to food allergies. B cells may also contribute to the development and exacerbation of food allergic responses through induction of allergen specific T cell activation, as they are capable of presenting allergen-derived peptides to T cells. On the other hand, B cells also contribute to the development of tolerance to food allergies. This occurs through production of allergen-specific antibodies of other isotypes than IgE such as IgA or IgG4 that may protect against food allergy. Although immunosuppressive functions of Breg cells have been described in several allergic conditions, it remains to be determined to what extent Breg cells contribute to the induction and maintenance of tolerance to food allergens.

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Many aspects of B cell biology in IgE-mediated food allergy remain uncertain. For example, how is immunological memory of allergen-specific IgE maintained, which anatomical site are involved in CSR to IgE and plasma cell differentiation of IgE-producing plasma cells, how do different exposure routes (e.g. skin or mucosal tissues) affect food allergen-specific B cell responses, what are the cellular responses of allergen-specific B cells to food AIT? The use of novel technologies such as single cell omics, BCR repertoire analysis and mass cytometry will enable us to significantly improve our understanding of these complex biological systems.

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Box:

Future Research Perspectives"

- Further elucidate the mechanisms that underpin the development and maintenance of the immunological memory of the IgE response.
- Identification of IgE-binding allergen epitopes and development of neutralizing antibodies that compete with IgE-allergen binding.
- Determine the role of regulatory B cells and effector B cells in food allergy and tolerance to food allergens.
- Further explore the consequences of direct versus sequential IgE CSR with regard to somatic V gene mutations and antibody affinity.
-

Box:

Recent Milestone Discoveries

- Identification of the role of TFH cells in induction of high affinity IgE production. [29]
- Detection of regional clonal foci containing IgE-switched B cells and plasma cells in the gastrointestinal tract, providing evidence for local CSR to IgE. [37]
- Evidence that development of long-lived IgE+ bone marrow plasma cells occurs upon chronic allergen exposure and is needed to establish serological memory. [60]

Author contributions

All authors contributed to drafting the manuscript and all authors approved the final version of the manuscript.

Acknowledgements

This work was supported by grants from the NIH (NIAID grants R01AI125567, U19AI104209 to S.D.B), the Swiss National Science Foundation (grants 320030–159870 and 310030_179428 to M.A.) and the Christine Kühne-Center for Allergy Research and Education (CK-CARE). Dr. Boyd reports personal fees from Regeneron, personal fees

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from Novartis, other from CareDx, other from Abcellera, outside the submitted work. The other authors have nothing to disclose.

Figure legends

Figure 1. Role of B cells in food allergy.

(A) **Food allergy development**: A major role for B cells in the development of food allergies is related to their capacity to produce IgE. This occurs when B cells are exposed to food antigens with the help from Th2 cell. They undergo class switch recombination to IgE and memory B cells. Then, these cells are differentiated into IgE-producing plasma cells that produce IgE antibodies, which bind to the high affinity IgE receptor on mast cells and basophils. Upon subsequent exposure to the culprit allergen, these IgE antibodies crosslink FcεRI, thereby triggering mast cell and basophil degranulation. The release of histamine, leukotrienes and cytokines induces allergic inflammation. (B) **Food allergy protection**: In contrast, B cells may also protect against food allergy. This process involves the induction of Breg cells, which produce suppressor cytokines such as IL-10, TGF-β, and IL-35. These anti-inflammatory molecules will suppress inflammation, promote IgG4 production and suppress IgE production. In addition, the IgG4 induction is also promoted by Tregs via the secretion of IL-10.

Abbreviations: FcεRI, Fc epsilon RI receptor; IgE, Immunoglobulin E; IgG4 Immunoglobulin G4; Th2, T helper 2; TGF-β, Transforming growth factor beta.

Figure 2. Allergic sensitization upon allergen exposure via the skin and gastrointestinal tract. Food allergens, upon ingestion, or contact with damaged skin, can be captured and processed by DCs. Epithelial cells produce alarmins such as IL-25, IL-33 and TSLP in response to external insults. These factors act on DCs, which migrate to draining lymph nodes, where they present allergen-derived peptides on MHC class II molecules to naïve T cells. These T cells (depending on co-stimulatory and cytokine signals) can differentiate to TFH or Th2. Th2 cells produce type cytokines such as IL-4, IL-5, IL-13 and IL-9 and play central role in allergic inflammation. TFH cells produce IL-21, IL-4 and IL-13 and induce IgE class switch recombination in B cells, plasma cell differentiation and allergen-specific IgE production. In GI related tissues such as stomach

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and duodenum, IgE class switch recombination in B cells, plasma cell differentiation and allergen-specific IgE production occurs in local tissue sites. The antigen-IgE complex with CD23 receptor on the intestinal barrier are directly translocated prior mast cell activation. Allergen-specific IgE antibodies that are released into circulation can bind to FcεRI molecules on mast cells and basophils. Subsequent exposure to allergen can result in mast cell and basophil degranulation.

Abbreviations: DC, Dendritic cells; IL, interleukin; TFH, T follicular helper; Th2, T helper 2; TSLP, thymic stromal lymphopoietin.

Figure 3. Direct and sequential class switch recombination (CSR) to IgE. (A) The immunoglobulin heavy chain (IGH) gene locus comprises different constant domains, of which IgM (encoded by Cμ) and, as a result of alternative splicing IgD (encoded by Cδ) are expressed in naive B-cells. Human B-cells can switch to IgE, either directly, or indirectly via an IgG or IgA1 intermediate, while murine B cells can switch to IgE, either directly, or indirectly via an IgG intermediate. During CSR, double strand breaks in the switch (S)-regions of the involved C-genes are generated, the intervening DNA sequence is excised and the Sμ fuses with the S region of the subsequent heavy chain isotype. In direct CSR to IgE, Sμ directly fuses with Sε, while sequential CSR to IgE involves at least one intermediate step. In the example illustrated here, sequential CSR occurs through an IgG1 intermediate that undergoes a second CSR event to IgE. The presence of a remnant of the Sγ1 region of the intermediate isotype in IgE-switched B cells, indicates that sequential CSR occurred. However, during the secondary CSR event, these S-region remnants can also be excised. Therefore, absence of S-region remnants of intermediate isotypes is not sufficient to conclude that CSR to IgE occurred through direct CSR. (B) Differences in mechanisms and outcome of direct versus sequential CSR to IgE.

Abbreviations: CSR, class switch recombination; IGH, immunoglobulin heavy chain gene locus; S, switch.

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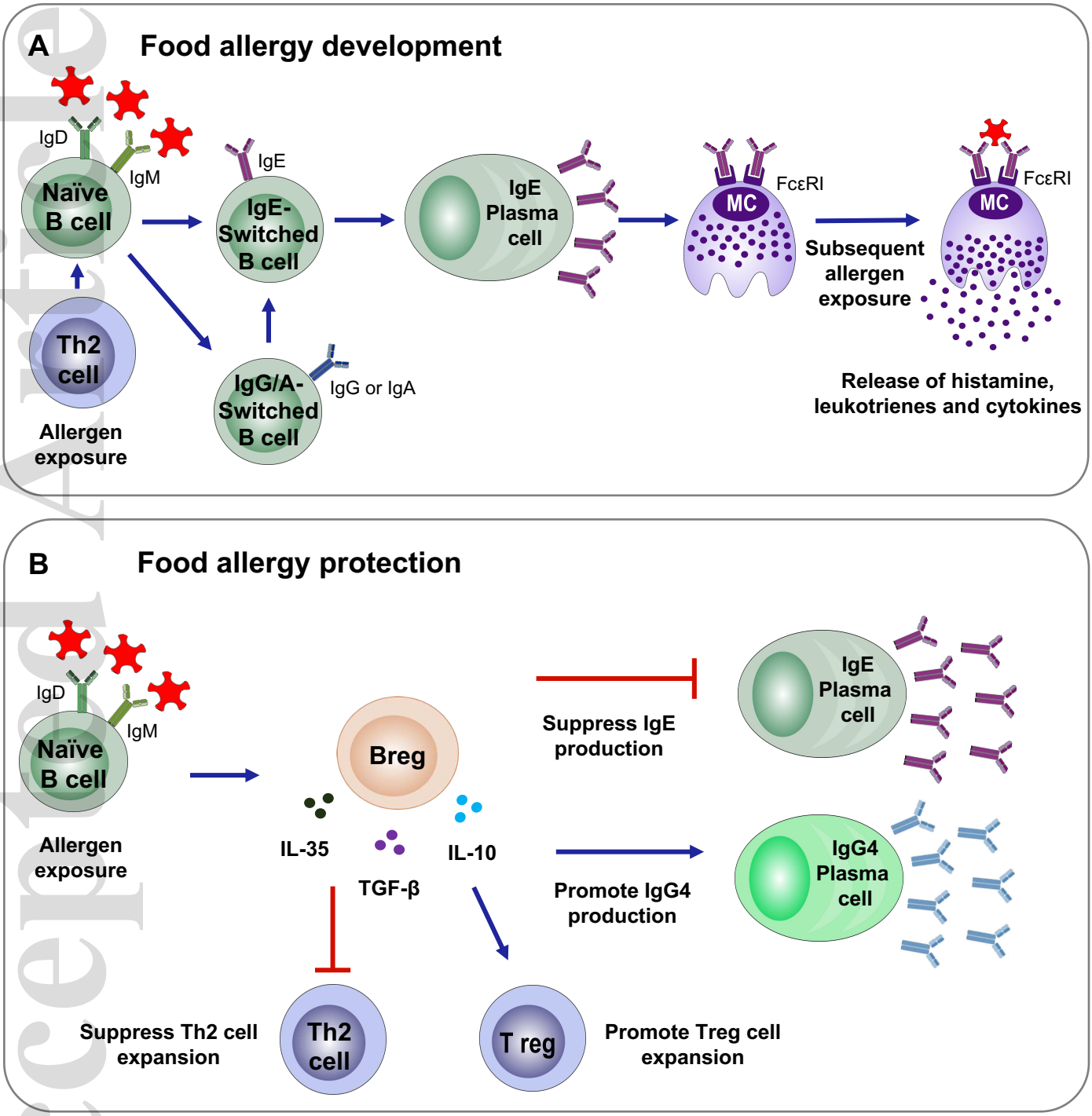
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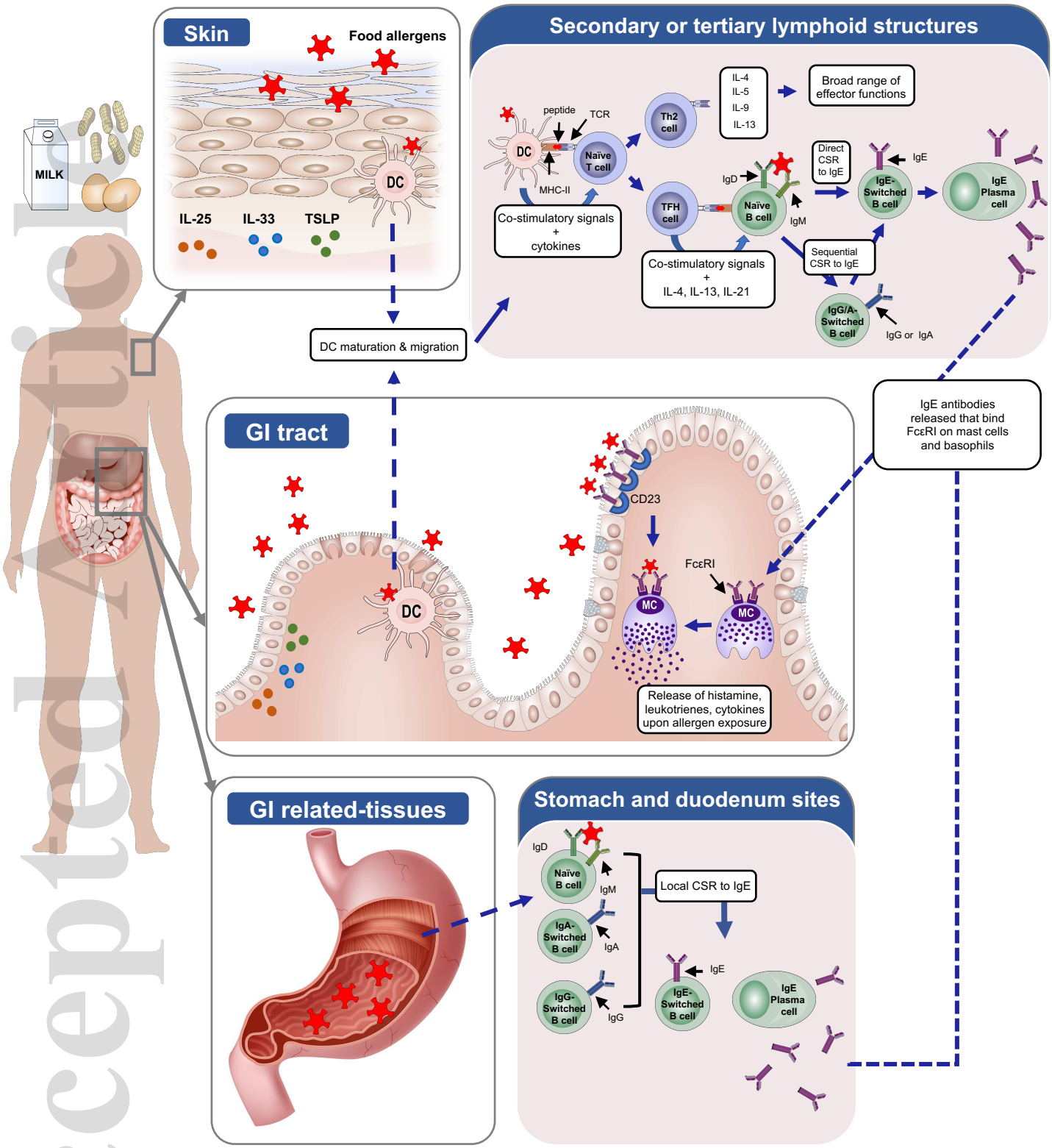
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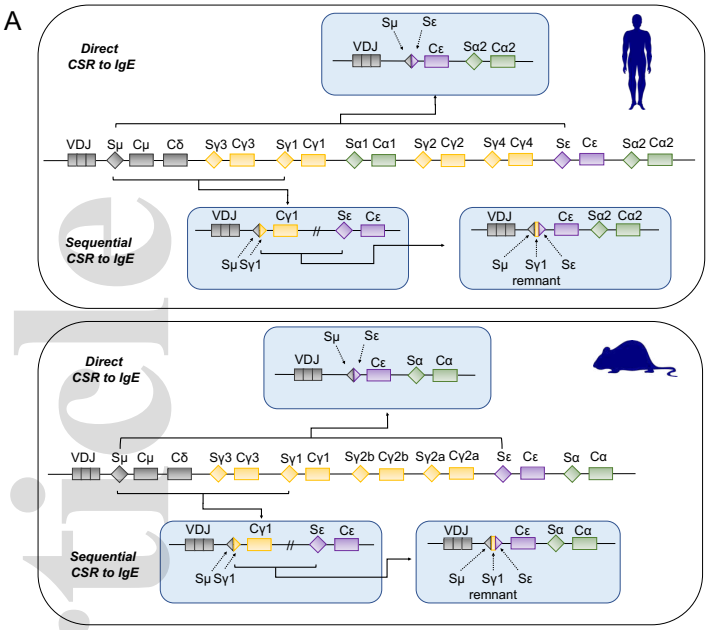
Figure 1

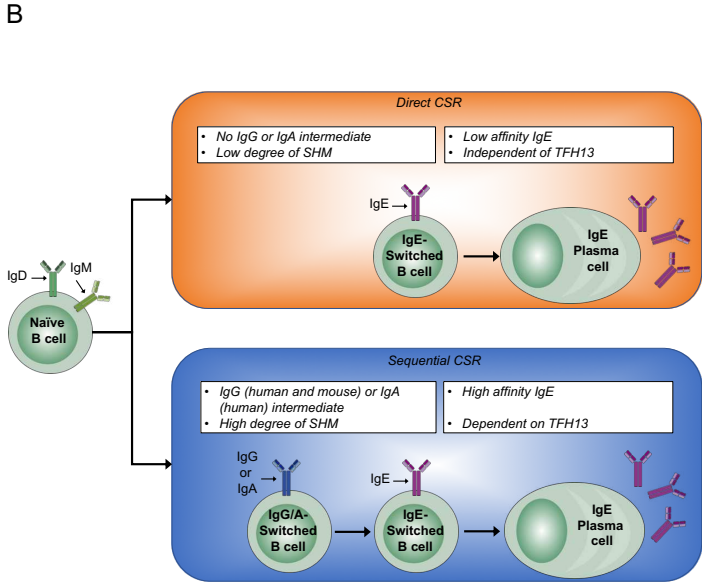


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Figure 2





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