Immunological tolerance for the diverse antigen binding domains of B cell and T cell receptors

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Plain language summary

White blood cells in the adaptive immune system have a special way of recognising almost any pathogen that can make you sick. While they are maturing, they make randomly generated detectors, each of which has the chance to detect a pathogen, like bacteria. However each of them also has the chance to detect things that belong in your body, this can make you very ill, so your body has ways to make sure that your immune system doesn't attack your own body. This requires your white blood cells to get in touch with your body's cells and their contents before they are fully grown and released into the blood. This can be a challenge, because they also need to come into contact with every single version of those randomly generated detectors. How does this happen? This review has put together an overview of the places where this could happen and has attempted to pinpoint exactly how this contact is established. The final conclusion is that there are adequate sources of these randomly generated detectors in the bone marrow, but in the thymus we are still missing a piece of the picture. Additionally, there are safety nets that can keep you healthy even if the body slips up. However, these safety nets might not be enough in cases where you get sick and the immune system is very active. Whether these safety nets are enough depends on the existence of the missing piece. Since we do not have any diseases related to these specific circumstances, we can conclude that the missing piece does exist and has yet to be discovered.

Abstract

The semi-random generation of lymphocyte receptor binding domains through V(D)J recombination creates a vast repertoire of receptors to combat pathogens. Establishing tolerance towards these receptors requires an equally vast repertoire of self-antigens. How is this repertoire established and what is its impact on the negative selection process? This review attempts to create an overview of self-antigen repertoires in the humoral immune system, with a focus towards B cell receptors and T-cell receptors. It establishes the possible roles and mechanisms of tissue restricted antigen regulators like AIRE, as well as the role of B-1 cells in this process. It also includes a brief overview of peripheral tolerance and its impact in this context. The final conclusion is that tolerance towards T-cell receptors seems incomplete, but the lack of relevant diseases indicates an as of yet undiscovered T-cell receptor repertoire.

Graphical abstract

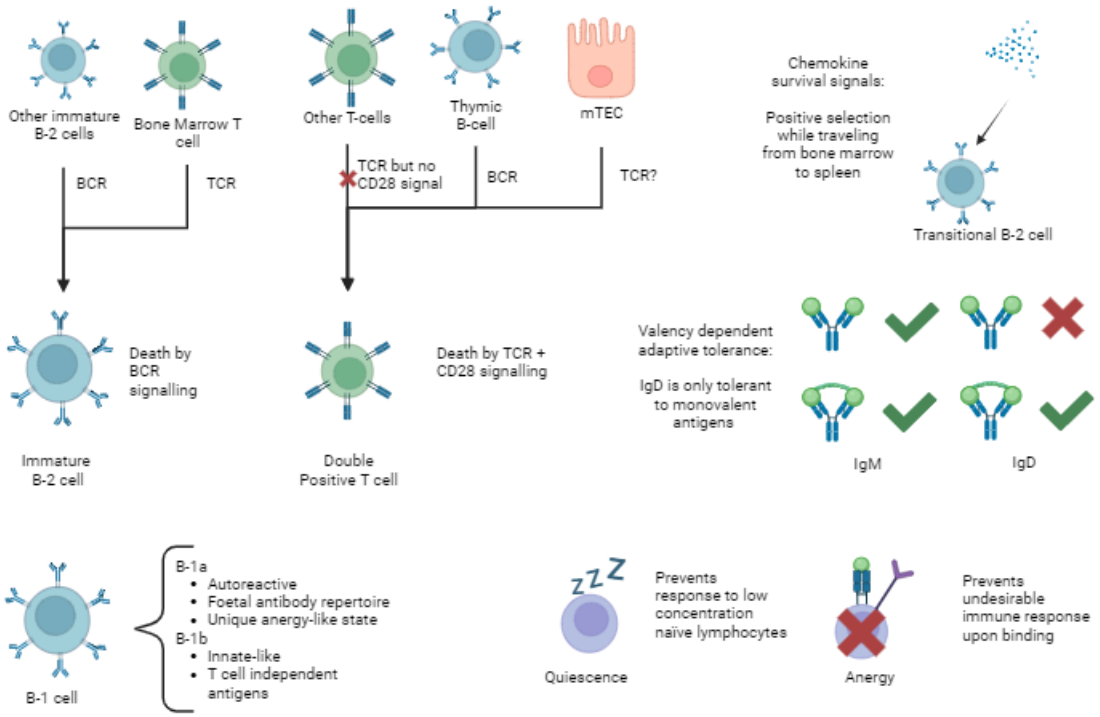

Image created in Biorender.com

Introduction

V(D)J recombination is one of the key mechanisms through which the immune system creates versatile binding regions. During lymphocyte development from hematopoietic stem cells, the genes encoding for B cell receptors (BCR) and T-cell receptors (TCR) are initially not in a state ready for transcription. The part of the gene that encodes for the antigen binding domain contains up to three regions: Variable (V), Diversity (D) and Joining (J), with the D region sometimes being absent depending on the gene [1]. Each of these regions consists of numerous individual fragments that each contain a recombination signal sequence on each end that allows them to be cleaved and ligated onto each other [2]. Before transcription, fragments of each region are picked at random and attached to each other, resulting into a translatable gene that has the potential to become a functional receptor chain. This process can be repeated several times and the total amount of different combinations can reach into the millions [3]. Furthermore, both T-cell receptors (TCR) and B cell receptors (BCR) consist of two adjoined chains. This allows for a great variety in binding domains on immune receptors [2,3].

However, such a diverse array of binding domains would inevitably contain binding domains that have an affinity for antigens that are not harmful, like most of the body's own cells. The first moment a BCR can recognise an antigen, approximately 70% binds to the body's own antigens [4]. This is reduced to 40% upon exiting the bone marrow [5] and only 3% survive to enter the mature population [6]. The body has a thorough selection process that prevents harmful autoimmunity in healthy people. When the immature immune cell binds too strongly to self-antigens, they go through a process called negative selection. During this process the receptors of harmful immune cells are either edited to be

unharmful or the whole cell is forced to go into apoptosis [3,7]. It is then of paramount importance that the body knows which antigens should be protected. This must mean that an antigen repertoire is present at the site of selection, to expose developing immune cells to antigens that they should not bind to. These mostly consist of the antigens in the lymphoid organs themselves, since they, like every other cell, express the key proteins required for survival. Furthermore, thymic medullary epithelial cells (mTECs) are known to express a transcription factor called autoimmune regulator (AIRE). This transcription factor is responsible for the expression of tissue restricted antigens (TRAs) that are not necessary for regular cell survival but are crucial for the functioning of organs other than the thymus. However, such a detailed antigen repertoire is as of yet not identified in the bone marrow. In fact, B cell selection being less tolerant could be an evolutionary advantage due to pathogens mimicking native antigens [5]. In addition to that, a subset of B cells called B-1a cells in mice [8],or CD20+CD27+CD43+ in humans [9], has been found that are positively selected for autoreactivity.

With this in mind, one could ask the question: Where and how are the V(D)J encoded domains presented? Due to the unique mechanisms of V(D)J recombination, it is difficult to consistently present every possible antigen binding domain that could be present on BCRs and TCRs. How then, is tolerance induced to the lymphoid cells? In this review we will go over the currently known mechanisms of antigen presentation for the purposes of negative selection of T cells B-2 cells and B-1a cells and establish where V(D)J encoded domains could be presented or otherwise participate in negative selection.

T cell

One way of presenting auto-antigens are mTECs. These cells line the medulla of the thymus, where single positive T cells migrate after passing through positive selection in the thymic cortex. TRAs in mTECs are regulated with two molecules: AIRE and Forebrain Embryonic Zinc Finger-Like Protein 2 (Fezf2). In mTECs 640 genes are induced by Fezf2 and 1553 genes are induced by AIRE, with 123 genes induced by both [10] and bioinformatics analysis further showed that 60% of TRAs are regulated by AIRE and Fezf2 [11]. Meanwhile 130 AIRE repressed genes were found while Fezf2 represses over 450 genes [10]. The same study also showed that the tissue expression of these TRAs differs, as Fezf2 expresses significantly more TRAs of the gastrointestinal tract and hematopoietic cells, while AIRE expresses more TRAs of the eye [10]. This shows that both AIRE and Fezf2 are important nonredundant factors in TRA presentation.

AIRE

There are three theoretical mechanisms for AIRE function: Stochastic binding and activation of TRA genes, promotion of expression through RNA elongation, and a regulatory role of mTEC development while they go through predetermined TRA expression stages.

The stochastic regulation by AIRE is supported by the lack of a specific binding region for AIRE, evidence that AIRE both activates and represses expression of certain genes, and that AIRE regulated genes are often found in clusters. The stochastic model proposes that AIRE binds to a random selection of genes within these clusters, causing some to be repressed while others are activated [12]. This model would allow for a large variety of TRAs to be expressed, with representation balancing out over several cells due to the stochastic nature. However other sources claim that AIRE does not function through transcription initiation and instead functions in RNA elongation [11].

While AIRE was found to bind to DNA in vitro [13] the binding domain that allegedly facilitated this binding, the SAND domain, was also shown to not be involved in recruitment to a plasmid promoter [14]. Furthermore, AIRE does not contain the conserved KDWK binding domain that other proteins with

the SAND domain do [14]. AIRE was also found to release a stalled RNA polymerase II at the transcription site of AIRE dependent TRAs by binding to Brd4 and starting a chain of events that lead to the recruitment of P-Tefb, which allows for RNA elongation to commence [15]. This leads to the current school of thought that AIRE does not bind directly to DNA but instead promotes gene expression by promoting RNA elongation. However the mechanism behind the target gene selection and exactly how it facilitates TRA expression is still uncertain, since it does not have a DNA binding domain. However, AIRE has not yet been tested for RNA binding motifs, which could explain selectivity to halted RNA polymerase II and the different binding motif compared to the KDWK motif in other SAND domains.

A completely different model is that of AIRE as a regulator of mTEC development. AIRE is expressed at certain stages of mTEC maturity. While other models reason that mTECs at this stage express AIRE in order to express TRAs, this model reasons that AIRE causes mTECs to reach this stage of development and that AIRE is not directly responsible for the regulation of TRAs [16]. The reasoning behind this is that certain TRAs that are not expressed in the absence of AIRE cannot be regulated by a closed locus, since adjacent genes are still able to be expressed. One example of this is the AIRE dependent TRA casein γ, as it is not expressed by Aire- mTECs, but casein α and κ which are directly adjacent to it are able to be expressed. On the other hand, AIRE is still required for casein γ expression while the locus is open. This point towards epigenetic means of regulation not being a sufficient explanation [16,17].

Within this line of thinking there are two contrasting models: AIRE promoting development and AIRE interrupting development. The former states that AIRE is a necessary step for immature mTEC cells to reach TRA expression stage, after which AIRE expression decreases and it enters apoptosis. The latter states that AIRE causes a differentiation in mTEC development, causing them to differentiate into separate cell type which goes into apoptosis in this stage [18]. Meanwhile, AIRE- cells develop to a similar cell type but instead of entering apoptosis after, they develop into a different stage, explaining the AIRE deficient mature mTEC population. These models combined with the observation that mTECs grow into structures resembling individual follicles in organoids [19] suggest that follicles could each have a separate states of differentiation. Roberto Perniola proposes a model where different thyroid follicles with different stages of differentiation (also called a mosaic structure) also present different kinds of predetermined TRAs, meaning that TRA expression is less stochastic than we think. [20].

Fezf2

The mechanism of Fezf2 has more in common with conventional transcription factors. While AIRE does not have a DNA binding site, Fezf2 is known to bind directly to promoter regions of Fezf2 dependent TRA genes [21]. Furthermore, Fezf2 does not bind to any AIRE dependent TRA genes and there are several AIRE independent TRA genes that depend on it [21]. Fezf2 dependent genes also showed a broader expression pattern in mTECs than AIRE. While AIRE dependent genes tend to cluster and be expressed on only a certain amount of mTECs at a time, Fezf2 dependent genes do not show this behaviour, suggesting that Fezf2 is not subject to mosaic clustering like AIRE is [10,22]. This shows that Fezf2 is a TRA expression regulator independent from AIRE.

Thymic B cells

Thymic B cells are a unique subset of B cells that reside in the thymus. In mice, they express CD5 and CD43 but lack CD11b, making them similar yet distinct from the peritoneal B1a cells discussed below [23]. Furthermore, they express high levels of MHCII compared to resting peripheral B cells. In humans,

only 50% of thymic B cells express surface CD5 [24]. They also express CD19 and CD20 but not CD27 and CD43, making them distinct from the human B1 cell population [9,23,25]. This shows that they are a unique phenotype present in the thymus. However, there is evidence that naïve B cells from the spleen can adopt the thymic B cell phenotype when exposed to local signals in the thymus [26]. This indicates that the thymic B cell population is not necessarily completely resident. In fact, replenishment with naïve B cells and a lack of thymic B cell precursors suggests that thymic B cell lymphopoiesis does not occur [26]. Additionally, the foetal bone marrow and liver cannot fully restore the previous population after lethal irradiation in RAG deficient mice [27]. This indicates that early in life, mice can replenish thymic B cell population without naïve B cells. However it is unknown if this is also the case for adult mice.

Thymic B cells have a unique and nonredundant role in central T cell tolerance. They are able to induce clonal deletion of T cells that bind to the antigens they present on surface MHCII [28–30]. Furthermore, if T cells in mice express a self-reactive TCR, an absence of B cells will cause incomplete central tolerance [23,27]. Some thymic B cells also possess the ability to express surface IgG and IgA without bacterial stimulus in pigs[31]. In mice, they are the first antibody secreting cells and also express IgE [32,33]. This indicates that they can uniquely undergo class switching without being activated by antigens. It has been theorised that this occurs to induce tolerance towards antibodies, as the timing of the class switching lines up with the age of mice where they stop generating anti-IgE antibodies [23,32]. Thymic B cells also notably express AIRE, though only 2.2% showed a punctate nuclear distribution compared to 26% in mTECs [26], but the function of AIRE in this case is unclear as AIRE induced genes were not enriched in TRAs [26]. The AIRE regulated genes in thymic B cells also show little overlap with those in mTECs [26], hinting at a unique function or repertoire. Overall thymic B cells show great promise for tolerance to the binding regions of BCRs due to the expression of a variety of immunoglobins and the possibility of novel AIRE functions.

One way of antigen presentation during T cell selection is often not mentioned, but for the topic of tolerance towards antigen binding regions it is of great importance: T cells are exposed to other T cells. This means that a developing T cell has the opportunity to bind to the MHCI molecules of another developing T cell. Consequently, a developing T cell is exposed to fragments of TCR bound to MHCI, which could trigger the internal negative selection mechanisms upon strong enough binding, this includes the antigen binding domain. However, it has long been known that the negative selection process requires costimulatory signals from CD28 [34]. Its ligands, CD80 and CD86, also known as B7-1 and B7-2 respectively [35], are not expressed on most T cells and only have a low level of expression on Treg cells [36]. This makes tolerance through T cell – T cell interaction unlikely. However, CD80 and CD86 are expressed constitutively in thymic B cells [26], giving further evidence that thymic B cells indeed play a role to immunoglobulins, but providing no answer how tolerance towards TCRs is established.

B-2 cells

Central tolerance for B cells is established in the bone marrow. Once a functional B cell receptor (BCR) is established, IgM expressed on the surface allows the cell to bind to antigens for the first time in its development. The immature B cell is then exposed to self-antigens, which will initiate receptor editing or cell death upon binding [7]. If an immature B cell is not reactive to self-antigen, it will express IgD alongside IgM and enter the periphery to finish maturation in the spleen. Detailed information about what cells express these self-antigens is scarce and often glossed over, however it is widely regarded to happen in the bone marrow before entering the periphery [37].

The negative selection of B cells is notably less strict than the process for T cells, since no AIRE analogue was found in the bone marrow [38]. This suggests that the antigen repertoire in the bone marrow does not include TRAs. Additionally, AIRE deficient patients still have a functioning central B cell tolerance [39,40]. This shows that B cell central tolerance is not AIRE dependent unlike T cells.

There are several reasons why a strict tolerance is arguably less necessary for B cells. One argument is that a tolerance system that is too strict would create gaps in the immune repertoire. Pathogens could mimic self-antigens that the body is tolerant to and avoid B cell immunity. This is already the case for viruses such as HIV-1, which can exploit gaps in the antibody repertoire to avoid the immune system [5]. The lack of TRAs in the bone marrow may be an evolutionary advantage that helps to avoid these gaps. Furthermore, B cell activation is dependent on T-helper cell activation, as it is impossible for B cells to develop into plasma cells without activation signals from a CD4+ cell [41], which have been selected for tolerance to TRAs. This allows for a tolerant immune system despite self-reacting BCRs. A B cell repertoire that has a slight affinity to self-antigens would therefore decrease the chance of an antigen dodging the humoral immune system by mimicking the host.

A recent idea in B cell selection is the idea of an adaptive tolerance, where autoreactive B cells are tolerant towards monovalent autoantigens but not towards polyvalent autoantigens [42]. This theory originated as mouse B cells that were thought to be anergic were actually still sensitive to polyvalent autoantigen, but not monovalent antigen. Furthermore, soluble monovalent antigen was competing with the polyvalent antigen. The adaptive tolerance model suggests an IgD dependent mechanism that allows cells to differentiate between monovalent and polyvalent antigen and would explain another threshold that needs to be passed before an immune reaction can be triggered. Furthermore, several autoantigens are a substrate for the enzyme transglutaminase, an enzyme that is highly upregulated during apoptosis and crosslinks its substrates, creating a polyvalent autoantigen. Autoantibodies against transglutaminase are also a specific marker for coeliac disease [43], with crosslinking of IgD itself likely being involved in the mechanism of action [44]. This distinction between monovalent and polyvalent antigens could play a major role in autoantibody generation.

Lastly, just as with T cells, B cells are exposed to each other's antigens in the bone marrow. Each BCR has the potential to bind to any other BCR. However, unlike T cells who require costimulation from CD28, BCR signalling is enough to induce apoptosis [45]. This means that B cell tolerance towards BCR binding regions and immunoglobulins as a whole could actually be induced by B cell – B cell contact alone. A similar mechanism could explain B cell tolerance towards TCRs. There is a population of memory T cells in the bone marrow [46,47], which hold a repertoire of TCRs that has been used in an immune response before. Thus, B cells can be tolerised for both surface BCR and TCR in the bone marrow.

B-1 cells

Studies have shown that 20% of the peripheral B cell repertoire is autoreactive even in healthy humans [5,48–51]. Part of this can be explained by T cell independent pathways like the B-1 cells identified in mice.

Evidence for self-sensitive B cells is present in the form of a natural antibody repertoire that is autoreactive. These are generally polyreactive antibodies of the IgM and IgG class. These are established early in life and are preserved over time [8]. Notably, they are not as random as regular antibodies as their utilisation of the V segment is not random, so they do not utilise the VDJ

recombination like regular B cells. This repertoire of antigens is theorised to be produced by the B-1 cell type identified in mice.

The key factor that distinguishes B-1 from the conventional B-2 cells is that they are able to produce antibodies through a T cell independent pathway. These cells primarily express low affinity IgM and they replicate through regular cell division instead of the highly sophisticated selection of B-2 cells. They also reside mostly in the peritoneal cavity before migrating to the spleen or intestines. B-1 cells are further divided into two subtypes: B-1a and B-1b, currently identified by their expression of CD5. B-1a (CD5+) cells are interestingly highly autoreactive, creating autoantibodies that are involved in apoptotic cell clearance. B-1b (CD5-)cells on the other hand react to T-independent antigens like oxidation specific epitopes involved in atherosclerosis [52] and many others reviewed in Vos et al. 2000 [53] and they can also form memory B-1b cells as reviewed in Alugupalli 2008 [54]. There was also a third subset discovered named B-1c, which did not express Mac1/CD11b, a previously used marker for B-1 cells [55]. However, it was later shown that CD11b- cells transition into Cd11b+ cells, so it is not a different cell type [56].

The development of B-1 cells is a controversial topic with multiple clashing models. The majority of the B-1 cell pool is generated during embryonic development and afterwards sustained through cell division. However, how the initial cell pool is generated is still unclear. The current model suggests that there is one progenitor for B-1a cells and B-2 cells. In this model, the progenitor can start premature Igk rearrangements and skip the pre B cell stage in development. To do this, a proliferative stimulus from the BCR binding to self-antigen is needed, causing positive selection towards autoimmunity [57]. This model explains the initial development of foetal B-1a cells with an increased tolerance towards deleterious BCR signalling. This occurs through the Lin28b-Let7-Arid3a pathway, of which Lin28b is known to promote proliferation [58]. This allows cells that would otherwise enter apoptosis through negative selection to enter the B-1a development pathway.

The B-1 cells found in mice are not directly translatable to humans. Humans do possess a polyreactive IgM excreting CD5+ B cell population, but it is not a unique population in the peritoneal cavity [59]. Furthermore, an abundance of CD5+ B cells was found in umbilical cord blood, but these turned out to be an intermediate developmental stage of B-2 cells rather than B-1 cells [60]. Rather, a functionally similar type of B cell has been found in umbilical cord and adult peripheral blood that expresses CD20, CD27 and CD43 as a unique phenotype [61,62]. CD5 expression seems unrelated to the B-1 like function of the human phenotype [63]. This has been theorised to occur due to an additional axon in human B cells called E1B (with regular CD5 being E1A), which encodes a truncated CD5 protein. Transcription of E1B inhibits transcription of E1A and leads to a decrease in surface CD5, however E1B transcription still leads to cytoplasmic CD5 [55,64]. How this affects the function of $CD20^+$, $CD27^+$, $CD43^+$ cell function compared to mouse B-1 cells is still unclear as the usage of CD5 as an identifying marker in mice makes it difficult to research its function [65].

Peripheral tolerance

Peripheral tolerance is a complicated system that prevents autoimmune reactions after initial lymphocyte development. The most straightforward regulation of autoreactive immune cells is to induce apoptosis and kill the cell. However there are several other less straightforward mechanisms at play as well. Some of these mechanisms occur in the autoreactive lymphocytes themselves, while others originate from secondary regulator cells, and yet others are originate from non immunogenic factors.

An example of peripheral tolerance intrinsic to the lymphocyte itself is the state of anergy. Mature T-lymphocytes require a double signal upon activation, one from the TCR and another from a coreceptor like CD28 [66–68]. If a T cell binds to an antigen without a costimulatory signal it can go into anergy, a state of inactivity and general loss of function, however it does not die. The lack of positive costimulation upon binding of the TCR causes the T cell to commence a different pathway which causes it to be hyporeactive and unable to divide. Furthermore, because it never received a costimulatory signal, the effector functions are never initialised and an immune reaction will not occur.

Where anergy is the active disabling of autoreactive T cells, quiescence is a passive inactivation of T cells where they are dormant until needed. Quiescence is the process by which naïve T cells are kept in a more dormant state compared to activated T cells. They are kept in their G0 state in the cell cycle (compared to G1 in anergy) [66] and they will not exit this state until a certain threshold has been reached. This ensures that low affinity binding of the TCR does not trigger the effector functions and only high affinity binding that clears the threshold will cause a immune reaction.

Death by neglect can be seen as a middle way between quiescence and anergy, yet more drastic. Where anergy occurs upon binding upon an antigen without receiving the proper positive signals and quiescence is a passive dormancy, death by neglect occurs when a T cell does not necessarily bind to something, but also does not receive positive selection signals. Rather than an arrest in the cell cycle like anergy and quiescence, the lack of positive stimulation triggers a pro-apoptotic pathway. This pathway promotes the expression of the Bax and Bak proteins, which push the cell into apoptosis through a Fas independent pathway [69–72]. This is a much more drastic method of selection compared to anergy, especially considering that anergic lymphocytes are directly autoreactive while only a fraction of neglected lymphocytes are [6]. However what exactly regulates whether the cell goes into anergy or death is a complicated matter of costimulatory molecule signalling that is not yet fully understood [67].

Ignorance is caused by barriers that physically prevent lymphocytes from starting an immune response. An example would be activated T cells being unable to enter the brain parenchyma. In healthy individuals, activated T cells are able to cross the blood brain barrier, but not the glia limitans. In individuals with multiple sclerosis, T cells are able to cross this extra barrier and cause the signature demyelinating effect that causes multiple sclerosis symptoms [73]. This is not a mechanism inherent to the cells itself, but more an effect of the environment that the T cells reside in. However, the circumstances combine with mechanisms such as the activation threshold of quiescence to prevent responses to a select repertoire of antigens that did not necessarily feature during central tolerance.

In B cells this process is less elucidated. After leaving the bone marrow, immature B cells move through the blood and enter the spleen. During this process they enter a transitional state, called T1 and T2 in mice. It is in between these states that a large portion of peripheral tolerance takes place, as a small amount of immature B cells leaving the bone marrow is still autoreactive[6]. These transitional B cells can also undergo the processes of anergy and apoptosis, but it is not yet clear whether this actually occurs in the spleen [74]. Furthermore, there is evidence that T1 cells still express the proteins necessary for receptor editing [74], something that T cells are not known to do in the periphery. As previously mentioned the mechanisms of tolerance for B-1 cells are still unclear, especially in humans. However, there is evidence that suggests that B-1 cells can enter a unique state of hyporeactivity that is distinct from anergy [75]. Death by neglect is also a large part of B cell selection as they move from the bone marrow to the spleen while still in development. Immature B cells require a positive selective signal to reach the spleen at all, if this is not received they die by neglect.

Currently there is no implicit positive selection signal known for transitional cells in the periphery, however the movement from bone marrow to spleen is known to be directed by the S1P1 receptor [76,77]. Although it is conventionally described as merely a chemokine and not a survival signal. Interestingly, there is a link between ceramides (which are structurally related to the S1P1 ligand) and the Fas receptor [78].

Regulatory T cells

Regulatory T cells (Treg cells) are a broad and adaptable cell type characterised by CD25 expression, lack of IL-7 and expression of CD4. Alongside these surface receptors, the transcription factor Foxp3 is a key protein in Treg cell function. While T-helper cells are able to initiate an immune reaction, Treg cells play a role in getting them to stop. There are several ways through which they can do this: One way does not require direct contact and is the excretion of immunosuppressive IL-4, IL-10 and TGFβ [79]. Other ways that do require cell-to-cell contact are by going directly to the lymph node where the immune reaction is triggered and prevent dendritic cells from recruiting more lymphocytes. Alternatively, the Treg cells can visit the inflamed tissue directly and inhibit effector T cells there [80].

Recent developments have also elucidated the many different functions a Treg cell can perform depending on their environment. Several different types of Treg cell have been discovered, which excrete cytokines reminiscent of other CD4+ cells: a Th1-like Treg phenotype that expresses Th1 markers like CCR5 and CXCR3 and upregulated Tbet, a Th2-like Treg phenotype that secretes IL-4 and IL-13 and has upregulated Gata-3 and IRF-4, and a Th17-like Treg cell that produce IL-17 and upregulate the transcription factor RORγt [79]. Besides these types of Treg cells, there are also other types that occur from other environmental factors, like how exposure to IL-10 causes a Treg cell to express STAT3 and inhibit Th17 cells specifically [81]. Furthermore, there is a wide variety of tissue specific Treg cells that each exhibit different behaviour depending on the tissue they inhabit. The mechanism behind this plasticity in function is still unclear. Though there is evidence that suggests that these modifications are reversible, there is also a possibility that each of these is a distinct lineage.

Regulatory B cells

Regulatory B cells (Breg cells, also known as B10 cells) are a population of B cells that excrete the immunosuppressive cytokine IL-10. They are a highly diverse phenotype and do not have a unique phenotype compared to other B cells apart from Il-10 production [82,83]. There are several mechanisms that can induce Il-10 expression in B cells, one of which is stimulation by TLR. However, TLR requires costimulation by other receptors as well to induce Il-10 expression, yet these receptors cannot induce IL-10 expression on their own. This could point towards an activation threshold for Il-10 production where lymphocyte activity needs to reach a sufficient level in multiple factors in order to trigger the B cell regulatory effect. This combined with the lack of a unique phenotype leads to the hypothesis that most, if not all, B cells are able to express regulatory functions without the need for a separate cell phenotype like a regulatory T cell. As for the functions of the Breg cell, due to the lack of currently known unique phenotype, these effects are synonymous with the effect of regular IL-10 release as reviewed by Mosser and Zhang [84].

Discussion

After reviewing the majority of tolerance mechanisms in the humoral immune system, it has become clear that there are not only opportunities to induce tolerance against BCR and TCR binding domains directly, but also that there are indirect methods of prevention should this tolerance never be

established. Immature B cells come into contact with both surface expressed TCR and BCR. The mechanism of clonal deletion allows them to go into apoptosis from BCR signals alone, allowing for negative selection to occur. Contrastingly, T cells require a costimulatory signal from CD28 upon TCR binding to enter apoptosis. This is mediated by the thymic B cell population, which expresses IgG, IgE and IgA alongside the CD28 ligands CD80 and CD86. However, T cells do not express these ligands themselves, so while a developing T cell does encounter TCR epitopes, negative selection does not occur. Therefore, T cells do not seem to gain tolerance for other TCRs through direct exposure.

Since the lack of tolerance is unique to T cells and specific to the TCR, it is possible that it is established through TRA presentation in the thymus. Hypothetically, if mTECs were to express (nonfunctional) TCR, then T cells would be selected against TCR binding, as mTECs do have the required signals for negative selection. Interestingly, AIRE gene regulation and mTEC expression studies have not yet included the TCR [85,86]. This hypothesis can be combined with the mosaic model of TRA expression [20] where mTECs grow in clusters and express different TRA repertoires which combine to make a comprehensive TRA repertoire. Different clusters of mTECs could systematically go through V(D)J recombination instead of randomly, which would create a comprehensive repertoire of TCR binding domains in line with the strict negative selection in the thymus relative to the bone marrow.

Since central tolerance mechanisms are not perfect, there is a possibility that this gap in central tolerance is mitigated by peripheral tolerance. T cell – T cell binding will likely result in anergy, as the costimulatory signal is not received. Furthermore, if tolerance to antigen binding domains is established through an as of yet unknown mechanism, the concentration of compatible antigens will be very low due to the random nature of TCR and BCR development. Each epitope is only carried by a single naïve lymphocyte, which is likely not high enough in abundance to break the quiescence threshold.

But what of the cases where the antigen binding domains are in high abundance, like when they are required for an immune reaction? If anergy is somehow circumvented, there is still the need for a T-helper cell and B cell to recognise the same protein as foreign. A B cell will need to phagocytose the VDJ antigen, digest it and then present it on MHCII. This would present not only the antigen binding region but also the constant regions of BCR and TCR, of which the latter does not have an established tolerance mechanism in T cells. The possible circumvention of this checkpoint by T cell independent antibody excreting cells like B-1a and B-1b is not of concern, as these cells transiently excrete antibodies of a limited repertoire. These would be present in the blood and would be part of the presented antigen repertoire during negative selection in B and T cell development. This would lead to the conclusion that T cell – T cell and B cell – T cell autoreactivity could occur as collateral damage during a humoral immune reaction.

Fortunately yet paradoxically, T cell deficiency following an infection is not a common condition, with the only candidate being AIDS after HIV infection [87]. However HIV targets the CD4 receptor directly, making it a primary cause of T cell deficiency rather than a secondary cause through infection. Following this observation it can be concluded that there is a cell in the thymus that presents TCR on MHCI and a CD28 ligand like CD80 or CD86, mTEC or otherwise.

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