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The five dimensions of B cell tolerance

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Abstract

B cell tolerance has been generally understood to be an acquired property of the immune system that governs antibody specificity in ways that avoid auto-toxicity. As useful as this understanding has proved, it fails to fully explain the existence of auto-reactive specificities in healthy individuals and contribution these may have to health. Mechanisms underlying B cell tolerance are considered to select a clonal repertoire that generates a collection of antibodies that do not bind self, ie tolerance operates more or less in three dimensions that largely spare autologous cells and antigens. Yet, most B lymphocytes in humans and probably in other vertebrates are auto-reactive and absence of these auto-reactive B cells is associated with disease. We suggest that auto-reactivity can be embodied by extending the concept of tolerance by two further dimensions, one of time and circumstance and one that allows healthy cells to actively resist injury. In this novel concept, macromolecular recognition by the B cell receptor leading to deletion, anergy, receptor editing or B cell activation is extended by taking account of the time of development of normal immune responses (4th dimension) and the accommodation (or tolerance) of normal cells to bound antibody, activation of complement, and interaction with inflammatory cells (fifth dimension). We discuss how these dimensions contribute to understanding B cell biology in health or disease.

KEYWORDS
ABO incompatibility, accommodation, antibodies, B lymphocytes, tolerance

1 | INTRODUCTION—B CELL TOLERANCE AT THE BEGINNING

In the late 1890s Paul Ehrlich and Julius Morgenroth probably first recognized what today would be called "B cell tolerance." Ehrlich and Morgenroth were investigating the properties of serum that seemed to connect specificity with effector functions in immunity to pathogens and toxins.[1] This subject was a matter of great controversy and it engaged practically all of the forebears of the new field of immunology.[1] Jules Bordet had reported that the serum of rabbits exposed to heterologous (guinea pig) erythrocytes acquired heightened ability to agglutinate and lyse fresh guinea pig erythrocytes.[2] Some believed that the agglutination and dissolution of erythrocytes by serum was analogous to the agglutination and killing of bacteria, but Ehrlich and Morgenroth considered that the impact of serum on heterologous erythrocytes was not biologically relevant since in normal circumstances rabbits are not exposed to guinea pig blood.

The biologically relevant question, Ehrlich and Morgenroth asserted was whether and how ones own erythrocytes are dissolved and whether this process relates in any way to protective properties of an immune serum. This question had a rational basis since internal bleeding generates hematomas that "dissolve" spontaneously over a period of weeks. It was to test the biological relevance of Bordet's observations for the formation and dissolution of hematomas that Ehrlich and Morgenroth conducted what in retrospect were the seminal experiments on B cell tolerance.[1]

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To model hematoma formation and to provide a maximum stimulus for generation of "lysins" Ehrlich and Morgenroth injected 800-900 ml of blood from one or more of nine goats into the peritoneum of five normal goats and they tested the sera of the injected goats for ability to lyse erythrocytes from the nine "donors" at various times thereafter.

The results of the experiment were striking. The serum of one goat, which had been injected with a mixture of blood from goats #1, #2, and #3, exhibited "strong" lytic activity against erythrocytes from goat #1 and goat #2, but notably less lytic activity against goat #3. The serum also exhibited lytic activity against erythrocytes from goats #4, #5, #6, and #9, but less against goat #8 and no lytic activity against erythrocytes from goat #7. Other goats treated with isogeneic erythrocytes exhibited distinct patterns, intensities, and kinetics of lytic activity against isologous erythrocytes; ultimately, 12 distinct isolysins were identified. However, in all of the experiments and in every condition tested no autolysin was ever detected.

Ehrlich struggled to explain how goats could generate highly specific isolysins without ever producing autolysins, since the side chain theory put forward in 1897 maintained that antibodies were receptors for nutrients and other substances that could benefit cells. In the end, they concluded that production of isolysins reflected "individuality" (a concept pioneered by Leo Loeb) and that the absence of autolysins reflected: "certain contrivances by means of which the immunity reaction is prevented from acting against the organism's own elements".[1]

2 | WORKING DEFINITIONS OF B CELL TOLERANCE

Although the term "B cell tolerance" is commonly used, reviews on the subject not infrequently omit a definition of B cell tolerance. For some, the definition of B cell tolerance may be so obvious that any statement of it would be unnecessary or even condescending. For some others, a definition of B cell tolerance might be "difficult to put intelligibly" as a famous American jurist explained omission of another definition, adding: "but, *I know it when I see it*".[3] For still others, straight-forward definitions of B cell tolerance inevitably fail to explain why all normal individuals have a preponderance of auto-reactive antibodies and B cells in primary antibody and B cell repertoires but lack overt manifestations of autoimmune disease, despite the many infectious and inflammatory challenges met during their lives.

If definitions of B cell tolerance are avoided today, definitions have been offered, courageously, in the past. Reflecting on the discovery of B cell tolerance as a distinct biological phenomenon, Brent [4] describes B cell tolerance as an acquired state of the immune system characterized by "specific unresponsiveness." This working definition advances well beyond the view of Ehrlich and Morgenroth, to envision B cell tolerance as: (a) specific; (b) acquired; and (c) a property of the immune system. These characteristics of tolerance remain are widely embraced if not central to the canons of modern immunology. Yet, each element of this definition is subject to challenge by observations made in recent decades. Below we describe some understandings about the nature of B cell tolerance and exceptions thereof and we offer several further "dimensions" that help align the canonical concept of B cell tolerance with normal physiology (Figure 1).

3 | B CELL TOLERANCE IN THREE DIMENSIONS

If Ehrlich's seminal experiments proved anything besides the existence of contrivances that prevent immunopathology, it was that antibody recognition could be extraordinarily specific and diversity potentially greater than theory provided. Ehrlich had already concluded that that antibody and antigen might fit each other like a key in a lock (a metaphor borrowed from Emil Fischer [5]). The idea that paratope binding to epitope more or less requires three dimensional fit still dominates teaching and investigation in immunology. The idea that the specificity of antibody and hence B cell recognition derives to a considerable extent from the three dimensional interaction of paratope and epitope traces to the lock and key model and that metaphor is still used to describe some antibody-antigen interactions.[6,7]

Despite the heuristic appeal of the lock and key model, interactions between antibodies and antigens, particularly paratopes with epitopes, are far more complex than the static, inert image a lock and key conveys. The three dimensional structures of antibodies do appear to fit some antigens, but forces never imagined in the original model govern the rate and extent or association and the permanence of binding. For example, electrostatic interaction and conformational motion govern association of some antibodies with antigens [8] and hydrophobic interactions and conformational flexibility the dissociation of some antibodies with antigens.[9,10] Induced fit of antibody and antigen likewise deviates from the classical concept. These complexities, however, need not detract from the idea that antibody and antigen combine in three dimensions.

What matters from a biological perspective is that the set conditions that enable antibodies to bind antigen with specificity and avidity must govern interactions between the BCR and antigen. If interactions of BCR with antigen departed from antibody interactions with antigen, B cell activation, affinity maturation, and receptor editing would generate some antibodies that are less effective in conferring protective immunity and some antibodies that inadvertently bind autologous cells or macromolecules. Thus, the generation and policing of tolerance by clonal deletion, anergy, and receptor editing depend on the fitting of BCR and antibody paratope with epitope in three dimensions, however complex as that fitting may be.

The three dimensional model underscores a potentially important challenge—effective induction of immunity and tolerance may depend on delivery to lymphoid organs of antigens faithfully representing the structure and surrounding microenvironment of antigen in microorganisms or in the body. This potential requirement is

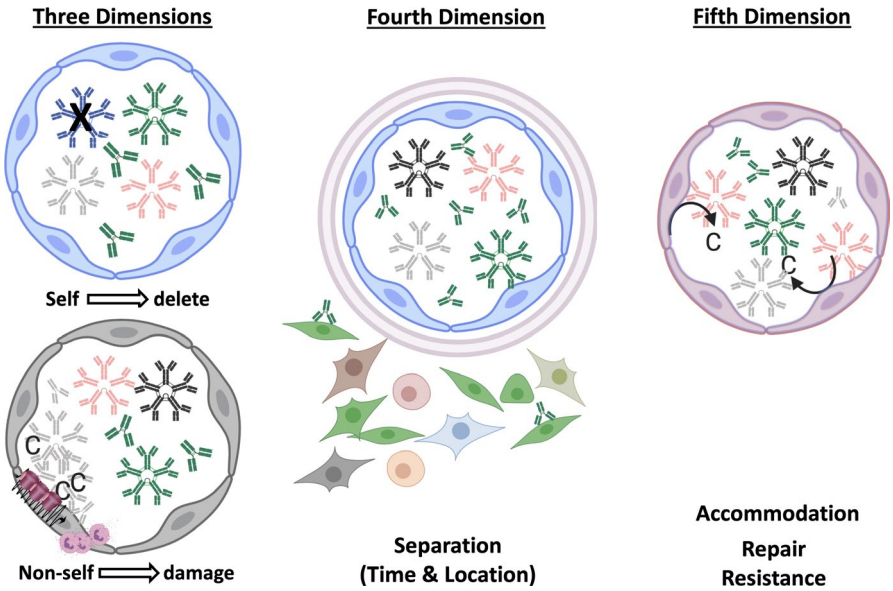

FIGURE 1 The five dimensions of B cell tolerance. B cell tolerance is classically defined as an acquired state of the immune system characterized by "specific unresponsiveness." Viewed in this way B cell tolerance is (a) specific; (b) acquired; and (c) a property of the immune system. Under this model what determines immune-pathogenicity is whether or not the B cell (and antibody) specificities exist (left panels). Because antibodies recognize native molecules in three dimensions this model accounts for the first three dimensions of tolerance. A fourth dimension posits that pathogenicity also depends on the kinetics of antibody production and on the separation of targets and blood vessels where most antibodies circulate. The fourth dimension explains why antibodies clearly contribute to rejection of solid organ allografts and not of tissues (middle panel). The fifth dimension represents resistance to immune mediated damage of cells. Tissues "adapt" to antibody mediated damage and to complement explaining why pathogenic antibodies do sometimes exist without causing disease. This process is called "accommodation" (right panel). C, complement. The circular arrow represents complement driven away from the cell surface; X, deletion

well-recognized as a key barrier to generating protective antibodies (especially broadly neutralizing antibodies) to HIV and some other infectious agents.[11] Accordingly, (i) simple polypeptide vaccines against HIV do not generate protective immunity, instead the antigen needs (a) correct 3-dimensional structure; (b) membrane Virus Like Particles (VLPs) to recapitulate the biochemical environment in which the antigen must be recognized, (c) other factors such as antigenic evolution also need to be taken into consideration.[11] Similarly, a requirement for delivery of antigens in native conformation and/or in the context of surrounding microenvironment to bone marrow or lymphoid organs has been given little consideration in the generation of tolerance.

Under the current model of tolerance, B cells that bind self-antigens engage regulatory mechanisms that reduce/abolish self-reactivity. These mechanisms consist of deletion (when the antigen is membrane bound [12]), anergy (when the antigen is in a soluble state [13,14]), or by receptor editing in which ongoing immunoglobulin (Ig) light chain gene recombination alters the B cell antigen specificity and in so doing rescues the auto-reactive B cell from deletion, [15-18] (reviewed in [19]). The concept of anergy was expanded by Fulcher et al[20] and by Cyster et al[21] who showed that anergic B cells in the periphery when in competition with non-auto-reactive cells, are excluded from lymphoid structures and gradually deleted. The consensus is that B cell tolerance results from deletion and receptor editing of developing auto-reactive B cells and from decreased viability and/or functionality of mature auto-reactive B cells (for a review [22]).

Immunity and tolerance to major blood groups in humans (blood groups A, B and O) provide insights into the precision, effectiveness, and limitations of tolerance in three dimensions. Synthesis of the blood group A and B antigens is catalyzed by glycosltransferases inherited as allelic variants.[23] All immune competent individuals develop antibodies against blood group A and/or B antigens they do not produce but have no antibody in the blood against the products of the transferases they express.[24,25] Individuals of blood type O produce neither group A nor group B saccharide and make natural antibodies against the corresponding antigens, while individuals of blood group A have antibodies against blood group B but not against blood group A.

Absence of antibodies in blood specific for autologous blood group antigens, might reflect self-tolerance. However, Karl Landsteiner, who discovered the major blood groups at the beginning of the 20th century, long maintained that absence of antibodies against these and other autologous antigens might simply reflect absorption of the antibodies from blood.[26] Therefore, identification and enumeration of B cells specific for autologous antigen could offer independent and potentially more incisive way to identify tolerance. Rieben and colleagues[27] used a limiting dilution technique to deduce the frequency of B cells secreting anti-blood group antibodies in the blood of human subjects and the results were striking. Approximately 1/10 000 produced IgM against allogeneic blood group A or B antigen but, <1/100 000 produced IgM that bound self-blood group antigens. The results are consistent with deletion

by one or another mechanism, the rare B cells producing antibodies against self-blood groups being recently produced or anergic.

Blood group incompatibility between a donor and recipient has been long considered a barrier to organ transplantation. Because blood group antigens are expressed also at high levels on endothelial cells and some epithelial cells, anti-blood group antibodies can recognize and potentially initiate injury of organs containing endothelium expressing foreign blood group antigens.[28] For reasons incompletely understood, natural immunity against allogeneic blood group antigens is not present at birth but develops in the first few months of life.[29] Therefore, transplantation of the heart in newborn infants offers an instructive view of the acquisition of immunity and tolerance.

In newborn recipients yet to develop mature B cells specific for the foreign saccharides in the graft, little or no antibody against the saccharide is detected once the B cell response matures and few or no B cells specific for the saccharide in the heart are detected in the blood.[30,31] These observations indicate that newborn recipients develop tolerance to the foreign blood group saccharide(s) (Figure 2). However, the recipients develop normal levels of antibody and normal B cell responses to foreign saccharides not present in their transplant indicating that tolerance is "specific". In mature recipients, tolerance to blood groups does not generally occur and recipients have varying amounts of antibody against the donor blood group detectable in blood after transplantation.

The experience in transplantation across blood groups illustrates an important limitation on analysis of tolerance. When abundant and/or persistent antigen generates immunity, the levels of specific Ab at various times may not reflect the intensity of the immune response and the absence of antibody after effective immunization could wrongly suggest the presence of tolerance. The underestimation of immunity and specious evidence that antibody production had been regulated or abolished was discussed long ago by Landsteiner.[26] Many others have observed a decrease in specific antibody in blood after introduction of antigen. This phenomenon occurs after organ transplantation.[32,33]

The most important limitation of the three dimensional model of tolerance is that it fails to explain how a primary B cell repertoire consisting mainly of auto-reactive B cells develops and persists.[34]

As the example of B cells specific for blood group antigens confirms, antigen specific B cells can be deleted during development. But clearly, for some abundant and readily available antigens, central deletion does not occur. Deletion or receptor editing of auto-reactive B cells might occur if BCR were to be stimulated in the absence of co-stimulation,[35] but potential for such stimulation should accumulate over time. We find it curious that delivery of co-stimulation in the context of tissue injury, vaccination, and other aspects of daily life does not activate auto-reactive B cells leading to affinity maturation and autoimmune disease. The B cells that produce antibodies that recognize tetanus toxoid and autoantigens, are potentially stimulated by tetanus vaccination and tetanus immunity but not autoimmune disease ensues.

4 | SPACE AND TIME: A FOURTH DIMENSION OF B CELL TOLERANCE

According to Ehrlich's concept of *horror autotoxicus*, some intrinsic facet of biological systems prevents autoantibodies from causing disease.[36] Immunologists generally maintain that B cell tolerance prevents autoimmune disease by preventing production of autoantibodies. Therefore, the study of the mechanisms that prevent autoimmune disease can shed light on mechanisms of tolerance.

If this concept appears reasonable, it evades important characteristics of the normal immune system. Normal individuals produce

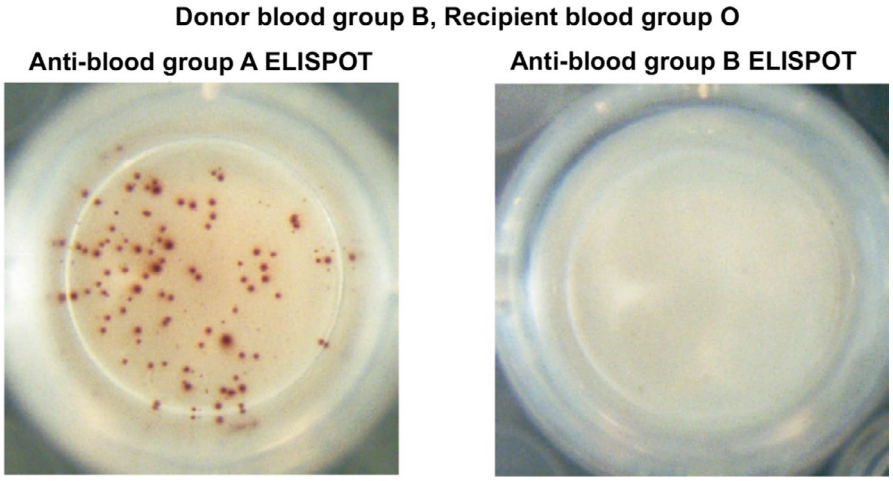

FIGURE 2 Tolerance by clonal deletion in ABO-incompatible transplantation. Newborn infants who receive cardiac transplants from donors incompatible for blood group A or blood group B commonly often develop tolerance spontaneously to the foreign blood group antigen in the transplant. The figure depicts an example. Antibody-secreting cells from an individual of blood group O who infancy received a cardiac transplant from a donor of blood group B is tested years later. The recipient has plentiful B cells that secrete antibody specific for blood group A (left) in ELISPOT but no B cells that secrete antibodies specific for blood group B (right). From Fan et al,[30] fig. 4, with permission of the publisher

at least some autoantibodies, including anti-DNA antibodies [37] and rheumatoid factor detectable in the blood [38] and yet individuals with those antibodies often manifest no disease (Figure 3). The autoantibodies in normal individuals are considered "natural" antibodies because production is not preceded by an eliciting event. Natural antibodies that are auto-reactive might be products of innate B1 B cells responding to bacterial products or might be evoked by autoantigens, much as natural isoantibodies are postulated to originate in one or both ways.[39] Although in both examples, there is no sensitizing event or evident co-stimulus, antibody production could be provoked by cross-linking BCR or by some occult, unrelated event that generates a second signal.

Natural IgM autoantibodies can be found in the umbilical cord blood in absence of exposure to any foreign antigens. They are polyreactive, bind several glycoproteins, phospholipids and glycolipids present in membrane receptors of autologous and allogenic cells. Auto-reactive natural IgM are encoded by germ line (non-mutated) variable region nucleotide sequences and lack N regions (Terminal deoxynucleotydyl transferase, TdT, inserted random nucleotides during repair of V(D)J junctions).[40] These properties are consistent with a fetal origin. Most natural autoantibodies are IgM (actually most IgM in normal sera are polyreactive and auto-reactive) but some monovalent IgM or auto-reactive IgG is detected.[41,42]

That IgM natural antibodies are protective in spite of their autoreactivity has been suggested by several authors. In one mechanism, dependent on the crystallizable fragment (Fc) of IgM, IgM limits BCR signaling by binding to the FcμR on B cells; in another, IgM controls the development of auto-reactive B cells in the bone marrow. Thus Nguyen et al showed that in the absence of FcμR, expression of IgM B cell receptor was increased augmenting tonic signaling, as a consequence.[43,44]

IgM autoantibodies control complement activation, do not mediate cytolysis at 37°C and scavenge C3 and C4 complement components. IgM inhibits TLR4 activation on endothelial and antigen-presenting cells, inhibits leukocyte chemotaxis by binding chemokine receptors and production of inflammatory cytokines. IgM clears self-antigens, preventing altered self-antigen-induced inflammation, ameliorates inflammatory conditions in murine models of complement-mediated glomerular inflammation, systemic lupus erythematosus, arthritis, and atherogenesis, and protects kidneys against ischemia-reperfusion injury and cardiac allografts against rejection. Thus, kidney and heart allografts in patients with high levels of natural IgM had better graft survival and lower incidence of acute rejection.[45-48]

FIGURE 3 Auto-reactive antibody-secreting cells in renal transplant recipients. B cells capable of secreting antibodies that bind to antigens on the surface of intact autologous cells are identified by ELISPOT performed using cultured fibroblasts as targets (cellular ELISPOT).[84] The figure identifies auto-reactive IgM secreting cells in a subject before kidney transplantation

What prevents auto-reactive antibodies from causing autoimmune disease? One might suppose the antibodies do not achieve the concentrations or the affinities of antibodies associated with autoimmune disease. But, this explanation is not fully satisfying for several reasons. First, germline encoded polyreactive antibodies can be driven by affinity maturation to generate antibodies associated with such effector functions as the neutralization of viruses and the formation of immune complexes.[40,49-53] Second, as we discuss presently, polyreactive antibodies do cause tissue injury and disease when conditions unrelated to the concentration and affinity of the antibodies allow pathogenesis to proceed.[54]

4.1 | Spatial separation of antibodies and antigen

Although natural autoantibodies potentially react with many different antigens, the targets available for binding in normal individuals and settings are quite limited. Most natural autoantibodies are IgM, which are located inside blood vessels. The potential targets are therefore limited to plasma proteins and components on the surface of circulating cells and endothelium. Under physiologic conditions the surfaces of cells are cloaked with glycocalyx that limits access of natural IgM and IgG antibodies to cell surface antigens.[55] Furthermore, the attachment of antibodies to cell surface antigens can be transient, as autoantigens in plasma cause bound antibodies to be shed.[56] Plentiful antigen in plasma, especially in monomeric form, limits or completely blocks the pathogenicity of circulating autoantibodies. To a similar effect, natural autoantibodies bound to components of the glycocalyx or to peripheral membrane proteins might be shed with the bound antigen, reducing or eliminating pathogenicity of antibody-antigen interaction.

The biological impact of natural autoantibodies changes profoundly when cell surfaces are damaged.[57] Physical injury, inflammation, and ischemia cause glycocalyx to be shed,[58] exposing neoantigens potentially to be recognized by natural autoantibodies.[59-63] Various neoantigens have been implicated.[57] Some antigens may be components of the inner plasma membrane trans-located as a result of injury, some may be fixed moieties on plasma membranes previously covered by glycocalyx. What distinguishes these antigens is that they are fixed to the cell surface and not apt to be shed with bound antibody. The binding of autoantibodies to these fixed antigens causes activation of the complement cascade and induces tissue injury. This injury could be considered autoimmune disease, as the specific binding of autoantibodies initiates the process. However, this type of autoimmune disease is not a reflection

of loss of tolerance, indeed tolerance was never present. Rather the autoimmune disease is brought about by exposure of fixed antigen that cannot be shed and not effectively blocked by circulating monomeric antigen.

The interaction of natural antibodies with autoantigens "fixed" on cell surfaces probably provides an important initial barrier to dissemination of microorganisms and toxins.[64] Individuals with selective deficiency of IgM are often found to have severe life threatening infection [65,66] some manifest autoimmune disease. What is important from the present perspective is that antibody-mediated tissue injury and autoimmune disease are not necessarily caused by absence of B cell tolerance and B cell tolerance in normal individuals does not preclude antibody-mediated tissue damage.

Spatial separation of antibodies and antigens also determines the pathogenicity of IgG. Perhaps the most instructive example can be drawn from heated debate about whether or not alloantibodies cause the rejection of transplants (see [67-70] for elaboration of the arguments). Put briefly, the discovery that allotransplantation almost invariably elicits production of alloantibodies [67] was seminal because it led to discovery and mapping of the major histocompatibility complex, as the key determinant of: (a) whether or not a graft would elicit alloimmunity; (b) whether or not an allograft would survive; and (c) a readily ascertained target of alloantibodies.[71,72] Yet, for more than a decade after discovery of the MHC, nearly all efforts to show that alloantibodies actually cause rejection of tumor and skin allografts failed.[73,74] Indeed, if alloantibodies had any impact on tumor or skin allografts, it was to prolong rather than to shorten graft survival.[75] It is now appreciated that alloantibodies can repair and protect grafts by one or more of several mechanisms, as we later discuss and elsewhere review [33,76-78]; however, the beneficial properties of alloantibodies are only observed when antibodies fail to exert full effector functions on grafts. What prevents alloantibodies from attacking tumor and tissue grafts with full effector function is the positioning of endothelium of the recipient between the alloantibodies and the graft. Thus, cell and tissue grafts residing outside of blood vessels are exposed to concentrations of alloantibodies and complement well below those in plasma. In contrast, alloantibodies directly contact the endothelium of organ transplants and hence may cause antibody-mediated rejection.

4.2 | Time

Another, potentially critical factor determining whether antibodies will spare or initiate injury upon binding to targets is the kinetics of change in antibody concentration. The rate of antibody production, delivery to the site of binding, diffusion and rate of removal and the condition of cells and tissues in which antibodies bind have considerable impact on the pathogenicity of antibodies. In the laboratory, the specificity and function of antibodies are usually tested by adding a known amount or dilution of antibody to a target cell or antigen. However, in biological systems, autologous cells are never exposed suddenly to "peak concentrations" of antibodies; rather concentrations of antibody increase more or less gradually, even in secondary B cell responses, and cells may respond in ways that limit injury.

The relatively gradual increase in antibody concentration is modeled by addition of sub-toxic amounts of antibody with or without complement to cells in culture. The response to antibody binding in this setting varies in part with the antigen: some antigens are modulated and some are shed, both of which processes could leave less antigen available for binding as the concentration of antibody increases. Cells exposed to sub-toxic amounts of antibodies also change in ways that may decrease the impact of further effector activity. For example, activation of C3 and C4 lead to covalent attachment of these proteins to cell surfaces, initially amplifying complement activation via formation of more convertase complexes. However, the bound C3 and C4 are ultimately degraded to catalytically inert C3d and C4d polypeptides that continue to occupy and thus to block sites with which activated C3 and C4 might react. This process may explain how some erythrocytes targeted by autoantibodies survive.[79] As another example, cells on which sub-lethal numbers of membrane attack complexes assemble undergo metabolic and structural changes that raise the threshold for cytotoxicity (reviewed in [80]). Thus, unlike Ehrlich's concept of *horror autotoxicus*, the production of autoantibodies is not necessarily inimical to survival or even to health.

On the other hand, microorganisms or transplants exposed to the blood of immune individuals have no opportunity to adapt to antibody binding and complement activation and relatively small amounts of specific antibody can be lethal for both. Using organ transplants as an example, reperfusion of an allogeneic kidney or heart with blood of a recipient who has antibodies specific for the transplant can lead to destruction of the transplant in minutes to a few hours, a process called hyperacute rejection. For this reason, potential recipients are screened by cross-match for antibodies against the donor of the graft.[81] On the other hand, donor-specific antibodies in concentrations that might very well have caused hyperacute rejection are not infrequently discovered in recipients with functioning transplants and in this setting cause no dramatic harm to the graft (the development of alloantibodies does however signal alloimmunity and is associated with the eventual occurrence of rejection [82]). Below we shall discuss some ways organs targeted by antibodies can evade injury, a condition called accommodation [80,83]; here, we use this example to emphasize that antibodies capable of inducing the most profound and dramatic immune-mediate injury do not necessarily cause pathology and disease. Accordingly, the absence of autoimmune disease cannot be taken as proof of immune tolerance.

The impact of the kinetics of immune responses has significance also for self-tolerance. B cells are continuously generated. During development and after reaching maturity, B cells with BCR (or BCR per se) that recognize self-antigens are eliminated. However, for reasons discussed above, the processes that censor the B cell repertoire remove many but certainly not all auto-reactive B cells. For example, when we devised a method for identifying and isolating allo-specific B cells, based on the ability of B cells to adhere to and secrete antibodies that specifically bind allogeneic cells, we found that every individual also have auto-specific B cells that secrete antibodies that bind autologous cells.[84] What prevents auto-reactive B cells from

inflicting injury? At any point in time, the absence of co-stimulation may prevent autoimmunity. However, at any given time infection or injury might generate the co-stimulatory signals. Under these conditions, then "tolerance" may reflect the ability of autologous cells avoid destruction when a small number of self-reactive B cells is activated.

The rate and conditions of antibody production and hence the apparent balance between immunity and tolerance may be subject to control by a highly polymorphic region of the genome. *TNFRSF13B* encodes the "transmembrane activator and CAML interactor" (TACI), a member of the TNF receptor superfamily. *TNFRSF13B* variants have been found to be associated with common variable immunodeficiency [85] and our early work appeared to support that idea. We found that the receptor encoded by *TNFRSF13B*, the "TACI" governs plasma cell differentiation and Ig production.[86] TACI is the receptor for BAFF and APRIL, which upon binding to TACI activates BLIMP-1,[87] the transcription factor that causes differentiation of B lymphocytes to differentiate into plasma cells [86,88] enabling production of large amounts of antigen-specific Ig.[89] There are however several arguments that counter the idea that *TNRSF13B* mutations are exclusively deleterious. Those are: (a) alleles that compromise protein function are expressed by 80% of humans and of these 98% are healthy; (b) research in our laboratory suggests that heterozygosity induces resistance to infection by enterobacteria (Cascalho et al; submitted); (c) broad surveys of *TNFRSF13B* genotypes in the population reveal an extraordinary degree of polymorphism (951 *TNFRSF13B* missense and only 383 synonymous, https://useast.ensembl.org/index.html), a high frequency of dominant negative alleles [90,91]; and (iv) according to the McDonald-Kreitman neutrality index *TNFRSF13B* is under strong positive selection pressure (in contrast to genes encoding HLA-class I which are under moderate purifying pressure).[92] These arguments suggest the possibility that *TNFRSF13B* diversity might be maintained by balancing selection.

We propose that *TNFRSF13B* polymorphisms in a population establish a continuum of immune responses varying between two extremes: (i) Low performing *TNFRSF13B* variants evoke antibody responses with high affinity at the cost of pathogenicity and autoimmunity owing to decreased natural antibodies, heightened complement activation, high affinity antibodies and enhanced cellular immunity; (ii) high performing *TNFRSF13B* variants evoke antibody responses of lower affinity with abundant natural antibodies protecting against immune-mediated injury in part by controlling complement activation, cellular immunity and inducing resistance to immune-mediated injury (accommodation). Common *TNFRSF13B* polymorphisms determine natural antibody production (IgM and IgA) which, in turn regulate complement activation and cellular immunity (De Mattos-Barbosa et al submitted). Thus, high performing TACI variants (a) may allow more complete or accurate censoring of autoimmune clones [93]; (b) may slow Ab production allowing targeted tissues to adapt; and/or (c) TACI may facilitate production of natural Ab (IgM) that may facilitate repair/healing.[57,94,95] Consistent with these ideas, many *TNRSF13B* variants are associated with aberrant B cell selection and with autoimmunity.[93] It is also possible that rapid generation of antibodies causes pathogenicity when associated with a relative deficit of natural Ab.

5 | ACCOMMODATION: THE FIFTH DIMENSION OF B CELL TOLERANCE

The original view prevailing today considers tolerance to be a property of the immune system.[22] This view led to seminal theories regarding self-non-self discrimination, clonal selection, etc.[96] The view also fueled many seminal discoveries concerning mechanisms of signaling and regulation of lymphocyte functions. Yet, as we mentioned above, the prevailing concepts of tolerance are at best incomplete. That is to say that normal individuals have many B cells (and T cells) capable of recognizing autologous antigens and appreciable amounts of auto-reactive antibodies in the circulation. Put in another way, the mechanisms of tolerance (clonal deletion, anergy, or receptor editing) of B cells that bind self-antigen fail to eliminate all auto-reactive B cells and auto-reactive antibody. Accordingly, we propose a fifth dimension of tolerance that originates from the interaction between the immunity targets and immunity. Thus, autoimmunity while present does not induce immunopathology.

The key concept of the fifth dimension is that absence of immune pathology results from actions originated both in the target tissue and in the immune system which balance protective and damaging responses. While aberrant primary development of "forbidden clones" or excessive signaling of B cells and/or T cells may cause autoimmunity and autoimmune disease, as often as not the inception of autoimmunity and immunopathology appears connected with immunodeficiency and hypogammaglobulinemia.[97-99] These observations might be taken to suggest, iconoclastically, that if the mechanisms that censor the BCR repertoire to avoid auto-reactivity functioned too well or too completely, *autotoxicus* would ensue. Various mechanisms have been postulated to explain how immunodeficiency might cause autoimmunity and we shall not undertake a critical review these here (reviewed in [100]). Rather, we shall discuss a mechanism we think merits consideration: "accommodation" or tolerance of tissues and organs to immune and inflammatory injury.

5.1 | Accommodation

The phenomenon of accommodation was discovered three decades ago when the deliberate transplantation of kidneys into ABO-incompatible recipients hesitantly entered clinical practice (see [80] for a more thorough discussion of these observations). Until the 1980s, transplantation of organs, particularly kidneys across ABO-blood group barriers (eg a kidney from an individual of blood group-A and/or -B transplanted into a recipient with iso-antibodies specific for one or both blood group antigens) was generally discouraged.[101-103] In up to 85% of recipients, ABO-incompatible kidney transplants either failed immediately to function or underwent rejection and failure within a few months.[104,105] Graft failure was understood to be triggered by the binding of anti-blood

group antibodies, which immune competent adults have [25] to foreign blood group-A and/or -B antigen,[106] which is expressed on endothelial cells.

Binding of antibodies to graft endothelial cells when the transplant is first perfused by the recipient can cause activation of complement which in turn causes a condition called hyperacute rejection that destroys the graft in minutes to a few hours (arguably, the most severe immune-mediated pathologic entity). Binding of antibodies to graft endothelial cells after the first hours or days causes antibody-mediated rejection, the pathology and molecular pathogenesis of which differs from hyperacute rejection.[76] ABO-compatible transplants were less likely to suffer early loss because the recipients are tolerant to A- or B-blood group antigens expressed in the graft and before transplantation potential recipients are screened by cross-matching to assure absence of the antibodies against HLA antigens expressed in the graft at the time of transplantation (development of these antibodies at later time however does cause antibody-mediated rejection).

In the 1980s, compelling need for organs for transplantation led some to transplant kidneys from donors of blood group A2, which is expressed at lower levels than the more common A1, into recipients of blood group O.[107] The trial was successful as the blood group incompatible transplants suffered fewer immediate and late losses. However, what was most important to the canonical understanding of B cell tolerance was that in most recipients anti-blood group A antibodies returned to the levels detected before transplantation.[107] Thus, in contrast with newborn recipients of cardiac transplants, discussed above, these mature recipients clearly did not develop tolerance (as classically understood) to foreign blood group antigens expressed the graft.

Reports of occasional successes in ABO-incompatible transplantation, especially the results mentioned above led others to explore whether deliberate removal of anti-blood group antibodies from the circulation and/or blockade by administration of antigen could prevent early (hyperacute) rejection and allow some ABO-incompatible transplants to survive and function thereafter.[80,105,108-110] The first trials were notably successful. Removal of anti-blood group antibodies (and perhaps also the incidental depletion of complement) from the circulation prevented hyperacute rejection and the transplants survived and functioned at rates approaching those observed for ABO-compatible transplants.

But what was most important for the present discussion was that at various times after transplantation, the recipients of the ABO-incompatible kidney transplants had antibodies specific for blood group antigens of the donor.[83,111-113] The success of these transplants could not be ascribed to the distinct properties of A2 antigen, as many of the transplants had incompatible A1 or B blood groups. Nor were these recipients tolerant, in the conventional sense as sometimes, indeed often, the recipients were found to have iso-antibodies directed against donor blood groups at levels equal to or greater than those measured prior to transplantation. Especially notable too was that the level of these antibodies was unrelated to the function of the transplant (see [80] and [113] for review).

The return to the circulation of antibodies specific for endothelial cell antigens in a graft without evident impact on the well-being of the graft could reflect one or more of several processes. It is possible that synthesis of the saccharide antigen might have changed, perhaps owing to generation of antibodies against glycosyl-tranferases or the antigen might have been shed or modulated after transplantation.[111,114,115] However, investigation of antigen expression before and after transplantation suggested expression had not changed.[83] It is possible, too, that antibodies produced after transplantation were different in affinity or effector functions.[115] No doubt the avidity of anti-donor antibodies in serum does change, but a decrease in avidity may well reflect preferential absorption of antibodies of the highest avidity to the graft.[32] Consistent with that possibility, antibodies bound to ABO-incompatible grafts were found to specifically absorb labeled blood group saccharide.[116] Thus, the antibody-antigen reactions found repeatedly to cause severe types of rejection evidently caused little or no injury and functional impairment in some of these transplants. We interpreted these findings to suggest that grafts might acquire resistance to injury caused by antibodies and complement and phagocytes, and named this change "accommodation" to denote that it was the graft and not the immune response that explained the absence of injury.[117]

Since the initial description, accommodation has been found to occur frequently in ABO-incompatible transplants and sometimes in xenografts.[76] Accommodation also occurs, although not without controversy, in organ transplants in recipients with antibodies directed against HLA antigens [84,118] as many transplant recipients are found to have antibodies specific for graft endothelium in the absence of antibody-mediated rejection. Accommodation is also postulated to benefit tumors and facilitate host defense against infection.[119]

The mechanism(s) that enable tissues and organs to sustain normal structure and function despite exposure to antibodies specific for surface antigens has been the subject of much investigation and some debate. There is general agreement that cells, tissues and organs targeted by antibodies must have at least normal if not increased ability to resist complement-mediated cytotoxicity.[120] Such resistance depends on expression of cytoprotective genes and activation of cytoprotective pathways.[121-123] However, more recent work indicates that if inhibition of cytotoxicity is essential for accommodation, it is also not sufficient. Accommodation requires repair of immune mediated injury and structural and metabolic changes that provide ongoing resistance to injury.[80,124] These changes transpire over days to weeks.[78,125]

The set of mechanisms that overcome antibody-mediated injury and resist subsequent injury are yet to be fully elucidated. We shall discuss one property in the section that follows. What is important for the present is that these mechanisms limit the impact of the most dramatic and severely toxic immune reactions on normal cells, tissues and organs. Accommodation thus has several implications vital to the understanding of B cell tolerance. First, if B cell responses generate antibodies that can mediate cytotoxicity but the antibodies cause little or no injury or dysfunction then

injury and organ dysfunction could offer misleading indices of tolerance. Transplant recipients who discontinue immunosuppressive therapy and do not suffer rejection are sometimes postulated to have spontaneously developed tolerance to their grafts.[126-129] Some of these "tolerant" recipients have antibodies in the circulation specific for antigens in the graft. Likewise, individuals with "autoimmune disease" with circulating autoantibodies sometimes have no overt manifestations of disease. These individuals may have accommodation rather than non-pathogenic B cell responses. Second, and still more important is the possibility that an untoward event might cause loss of structural integrity and metabolic changes that prevent injury caused by ongoing autoimmunity or alloimmunity. Yet, investigation of autoimmune- and alloimmune-mediated disease usually focuses on changes in the immune response rather than susceptibility to injury.

5.2 | Clearance of antibodies

Investigation of B cell responses and B cell tolerance usually relies on the assay of circulating antibodies specific for the antigen or set of antigens of interest. If the antigen of interest is uncertain, antibodies against other antigens can be assayed as surrogates. Transplantation provides a clear example of the opportunity and challenges regarding B cell tolerance. Tolerance to the graft is the clinical ideal outcome, as the tolerant recipient is freed of the requirement for and side effects of immunosuppressive therapy. Indeed, as mentioned above, some transplant recipients are believed to have spontaneously acquired tolerance to their graft, as cessation of immunosuppression does not lead to rejection.[126-130]

One might expect that tolerance to a transplant would be readily identified. In experimental systems, transplantation reliably generates immunity to allogeneic major histocompatibility antigens. Antigens encoded in the major histocompatibility complex are highly immunogenic (immunogenic enough that MHC was originally identified and mapped using allo-antisera) and the antibodies elicited by transplantation are quite specific (allo-antisera for laboratory use are generated by transplantation). Moreover, assays for anti-HLA antibodies and donor-specific antibodies have been perfected over decades and standardized for application in clinical laboratories and overwhelming evidence indicates that alloantibodies provide the most sensitive and specific predictor of alloimmunity and rejection.[81] Nevertheless, the absence of donor-specific antibodies in the blood of a transplant recipient is not taken to indicate tolerance and no approach yet devised reliably identifies B cell tolerance to a transplant antigen.

What might well pose the preeminent obstacle to identifying B cell tolerance to self and to transplants is that normal cells and tissues potentially can absorb enormous amounts of antibody from blood [32] therefore, absence of antibody in the blood against a given cellular antigen could reflect absorption rather than specific non-production. We have discussed this problem in various contexts,[80,123] but we are scarcely the first to do so. Landsteiner [26] considered the absorption of antibody to antigen and removal from the circulation to be key hurdle to understanding the governance of antibody production.

Work in transplantation, where the concentration of antibodies specific for foreign antigens can be ascertained, confirms the extent of the problem. In ABO-incompatible organ transplantation, the assay of antibodies specific for foreign blood group antigens often reveals that reperfusion of the organ causes substantial if not full depletion of iso-antibodies (see [80] for review). Indeed, perfusion of an isolated organ effectively depletes natural antibodies directed against xenografts.[131] Indeed, the main limitation to depletion of antibodies in this way is that activation of complement causes constriction of and injury to blood vessels, in turn limiting the period of perfusion to about 30 min in this extreme model in which regulation of complement is impaired.[132] The implications for investigation of B cell responses and tolerance in transplantation and other conditions in which antigen is plentiful are that antibodies of the highest avidity and antibodies specific for the most plentiful antigens may be preferentially depleted by binding to cellular antigens, leaving in the blood antibodies of lower avidity and/or directed against scarce antigens.

There is another facet of the phenomenon of antibody depletion that may be pertinent to B cell responses and to tolerance. The ability of cells to remove antibody from blood or extracellular fluid varies in the part with the antigen. Investigation of cells in culture reveals that antigens expressed on plasma membranes vary in response to bound antibodies—some antigens appear inured to bound antibody and expression continues,[56,133] other antigens are modulated and the bound antibodies are either metabolized or released intact from the cells.[134,135] However, antibodies bound in vivo are more robustly depleted and metabolized, suggesting that cells in culture may fail to fully represent the fate of antigen and antibody. We suspect that the difference is likely owed to the impact of complement. Complement activation on cell surfaces triggers endocytosis, which removes complement complexes and potentially bound antibody.[136]

The process of antibody clearance has important implications for understanding of B cell responses and tolerance. Bound antibodies that activate complement might be more readily and fully cleared than antibody that does not activate complement or antibodies bound when complement is inhibited. Thus, autoantibodies or alloantibodies that bind to healthy cells and activate sub-lytic amounts of complement might be effectively depleted and assays of the blood might thus suggest that little or no antibody has been produced. On the other hand, if cellular functions are impaired or if cells are injured, the ability to take up and metabolize antibody decreases and autoantibody or alloantibody levels in blood might increase. These scenarios are the opposite of what is usually envisioned as the immune pathogenesis of disease and it is not clear whether and when this series of events can occur. Surveys of recipients of kidney transplants reveal that donor-specific antibodies often appear after rather than before episodes of rejection are detected,[33] suggesting the possibility that antibody absorption impaired detection of immunity until the graft had been injured by rejection. This process is also pertinent to accommodation, as

resistance to complement-mediated injury probably depends in part on efficient clearance of complement complexes from cell surfaces.

6 | CONCLUDING REMARKS

The prevailing view of B cell tolerance as an active process that obviates or at least minimizes specific recognition of self has had immense value. This view fueled investigation of the cellular basis of immunity and clonal selection as a process that enables robust protective responses that spare autogenous cells (for a review [22]). The original view of B cell tolerance "in the three dimensions," as we put it gave impetus to understanding how co-stimulation and germinal center reactions regulate the specificity of B cell responses. Yet, perhaps inevitably, models focusing on individual physiologic systems can over-simplify and in some cases distort understanding of processes that support well being in vivo. The original view of B cell tolerance may exemplify that problem.

Autoantibodies and the B cells that produce autoantibodies, while not understood as fully as B cells that produce antibodies directed at foreign antigens, confer obvious benefits in host defense, repair of injury, and immune regulation. Whether the existence of self-reactive B cells reflects "leakiness" or an evolved bypass of the processes that censor lymphocyte repertoires cannot be addressed today. What must be countenanced, however, is that all healthy individuals have auto-reactive antibodies and many individuals with inherited immunodeficiency diseases lack these antibodies, in part or entirely. Accordingly, any consideration of how tolerance influences well-being must allow that depletion of all auto-reactive B cells would leave an individual at greater biological jeopardy than would imperfect or incomplete depletion of auto-reactive B cells.

To account for the contribution of autoantibodies to overall fitness, we discuss two further dimensions of B cell tolerance. One dimension focuses on the localization of antigens recognized and the period of time during which antibodies are produced. This dimension views natural autoantibodies as intrinsic to the normal immune system. Natural autoantibodies may or may not bind infectious organisms but they reliably bind injured cells, recognizing antigens that are either hidden or displayed in ways that binding of antibodies does not disrupt tissue vitality and function. When infection or injury occurs, vascular and lymphatic surfaces appear immediately to display epitopes recognized by natural antibodies. Although natural autoantibodies can have distinct properties, such as poly-reactivity and encoding by germline V region gene segments, understanding how the antibodies contribute to well-being requires more attention to the nature and location of the antigens recognized. Such attention will hopefully explain how the same antibodies that confer protection or initiate repair in some settings induce tissue injury in others.[57] The location of the antigen on vascular cells facilitates the containing of infectious organisms and toxins with a segment of a tissue; but, it also allows natural antibodies to participate in the ischemia-reperfusion injury that afflicts entire transplants.[64]

Since the physiologic actions of natural IgM and IgG autoantibodies are in part confined to surfaces of endothelium and circulating cells the B cells that produce those antibodies need not respond to antigen by massively increasing production. However, excess of natural autoantibodies potentially could induce injury. Therefore, regulation of these B cells differs from the regulation of B cells that produce elicited IgG. Nevertheless, regulation does ultimately employ clonal deletion, selection and/or receptor editing.[30,137]

Yet a fifth dimension of B cell tolerance is put forth to allow for the possibility that some autoantibodies will escape control and potentially induce harm. Such noxious autoantibodies might be the products of B cells that escaped control by the conventional (three dimensions of tolerance) or of B cells that produce natural autoantibodies but for some reason do so in excess. What we see more or less universally in transplantation and perhaps might see in normal physiology is that excess or untoward antibody binding to autologous cells does not inflict irreversible injury because the cellular targets can adapt to antibody binding, to activation of complement, opsonization and perhaps other noxious events by acquiring ability to repair damaged surfaces and resist cytotoxicity. That is, tissues can become tolerant of immunity as immunity is not entirely tolerant to self. We call this condition "accommodation."

Since all normal individuals have circulating autoantibodies and since complement is continuously activated in normal blood (both by alternative and classical pathways), we suspect the normal, baseline state of endothelium and circulating cells includes some measure of accommodation. On the other hand, the posture of accommodation likely subsides in endothelial and hematopoietic cells stored and cultivated outside normal blood and that makes the cells more susceptible to acute injury. We have some understanding of how accommodation is induced and the changes it embodies but rather little about how it subsides. However, under conditions of infection, accommodation probably subsides quickly, allowing complement and leukocytes to sequester organisms and toxins in vascular segments. A particularly dramatic example of the subsiding of accommodation might be the newly transplanted organ. Only during the first day after transplantation is an organ susceptibility to the dramatic and devastating condition known as "hyperacute rejection" induced by antibody binding and complement activation. After a few days, transplants acquire at least some resistance so that while antibodies and complement induce rejection (antibody-mediated rejection), the pathology and course are distinct and notably less dramatic and severe.

The perspective this fifth dimension brings raises the possibility that autoimmune disease or transplant rejection might occur in some cases when accommodation subsides or fails. Whether or not and how often that pathogenic mechanism applies is unknown; but nonetheless important because it opens a window onto new therapeutic targets and strategies. And, regardless of whether one prefers the long-standing definition of tolerance or a broader view that includes accommodation, the perspective reinforces the need to study B cells and the targets of antibodies and not just antibodies to achieve fuller insight into immunity, tolerance, and disease.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest with the contents of the manuscript.

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