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B CELL/ANTIBODY TOLERANCE TO OUR OWN ANTIGENS

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1. ABSTRACT

The lymphoid system normally mounts damaging responses to infectious pathogens while avoiding equally damaging responses to self. A notable number of antibodies to self antigens are formed but normally remain at levels below the damaging threshold, only temporarily rising to damaging levels during protective responses against infectious nonself. Many mechanisms regulate the level of autoantibodies and anti-self B cells including deletion, anergy, ignorance for antigen, receptor editing, coinhibition, competition for resources to sustain B cell responses, and apoptotic denouement of damaging responses following the ejection or containment of foreign invaders. While infectious events may encourage immune responses to self antigens, infectious events tend also to strengthen regulatory mechanisms. When regulatory mechanisms do not function properly, abnormal damaging responses to self antigens may occur. While defects in a single regulatory mechanism may result in autoimmunity, this eventuality usually happens only on permissive genetic backgrounds; this indicates that weakness in other regulatory mechanisms may be necessary to result in the emergence of damaging responses to self antigens. The immune system and its regulatory mechanisms are not simple, as one would expect of a homoeostatic process that also has the ability to expand enormously when challenged and to contract rapidly when threats pass. These processes that avoid damaging anti-self B cells are much more complicated than that envisaged in standard two signal models. Simple signals through the B cell antigen-receptor probably encourage B cell survival and receptivity, while other signals (costimulatory or coinhibitory) promote B cell stimulation or non-stimulation/inactivation.

2. INTRODUCTION

One can make two statements with equal conviction. The first is that B cells are tolerant to self antigens. The second is that B cells are not tolerant to self antigens. The first statement recognizes that immune systems that inflict continued damage on host tissues bearing self antigens would be maladaptive, or, as Ehrlich and Morgenroth put it, dysteleogic (1). The second statement takes into account that anti-self B cells are by no means rare and, with a little effort, can be activated to produce anti-self antibodies. Because of these two differing viewpoints, B cell tolerance is hard to define in one simple dimension. In terms of the subject of this review, the best definition that this reviewer can come up with is the absence of damage of self cells and tissues by antibodies, the product of B cells. This leaves the mechanisms of B cell tolerance undefined, or better, defined but not placed any order of importance.

Up until recently, immunologists felt duty-bound to state maxims with no shading. Anti-self B cells are deleted at an immature stage due to a simple negative signal delivered through the B cell antigen-receptor (BCR). To attain activation and antibody production, mature B cells require a signal via the BCR and a second signal from T cells that recognize peptides derived from the same

antigenic particle recognized by the B cell (2). Some immunologists even suggested that the BCR signal did not represent a B cell activation mechanism but a mechanism for preparing antigenic peptides for presentation to T cells that then delivered the truly activating signal for B cells (3). While this simplified B cell activation, it did not take into account that B cells were affected in various ways by T-dependent antigens in the absence of T cells, but failed to generate complete immune responses. The desire for simplicity, so-called minimal theories, drove immunology to the point that statements about immune responsiveness and tolerance were based more on faith or loyalty to a school than on observations.

Bringing observations into our understanding of B cell responsiveness and tolerance has not been easy. It is difficult to detect the small proportion of B cells caught in the act of generating an immune response or becoming tolerant, and to follow the behavior of this small proportion of B cells after they have undergone clonal selection or clonal inhibition. It is like trying to get a picture of a busy city street, in which all people and cars are moving, with a very slow film requiring an exposure time of hours; what shows up in the photo is an empty street. We can only observe what our technology allows us to observe. This means that the interpretations of our less than perfect observations must be well considered.

Much of the current observations regarding B cell responsiveness and loss of responsiveness involve the use of transgenic models in which immunoglobulin heavy and light chains are placed in stem cells. Because all B cells have the transgenic heavy and light chains with a defined specificity for a nominal antigen, one can study B cell development in the absence of antigen or in the presence of antigen, usually as another transgenic molecule under various forms, as a neo-self antigen. The problem with transgenic experimental models is that they are anachronistic in that they express self-reactive BCR at developmental stages when non-transgenic B cells do not. Also, the transgenic antigen may not mirror completely the host antigen. While most transgenic models have been used extensively to bolster the claim for the primacy of deletion, some transgenic models indicate that anti-self B cells are not affected by the presence of self antigen (4) or that 'self-antigen' can positively select for lymphocytes with anti-self specificities (5). Another experimental model utilizes superantigens that activate a large proportion of lymphocytes (6) via receptors other than the BCR; how this form of activation relates to that afforded by classical antigens via the BCR is a matter of considerable debate. Some B cell superantigens do operate via the BCR (7), but again the process may be different than that mediated by classical antigens. Another polyclonal approach is the use of F(ab')2 anti-BCR antibody that stimulates a large proportion of B cells, but this approach can only work in agammaglobulinemic systems (for anti-BCR antibody to bind BCR and not to soluble immunoglobulin) but with normally responsive B cells; these conditions require the use of agammaglobulinemic *in vitro* systems in which the architecture of the lymphoid system has been disturbed or obliterated.

This review will follow recent ideas regarding the mechanisms for B cell/antibody tolerance to self antigens, and how these ideas have been influenced by experimental models that may or may not be reflective of normal host antigens and normal BCR to these antigens. Ehrlich described these mechanisms for preventing damaging immune responses to self in the following way (1) – "the organism possesses certain contrivances by means of which the immunity reactions, so easily produced by all kinds of cells, is prevented from acting against the organism's own elements and so giving rise to autotoxins. Further investigations made by us have confirmed this view, so that one might be justified in speaking of a 'Horror autotoxicus' of the individual. These contrivances are naturally of the highest importance for the individual." The term 'Horror autotoxicus' was for Ehrlich the solution to the problem of autoimmunity (fear of autoimmunity requiring contrivances), not the problem (horrible autoimmunity). Today we appreciate the extent of the problem, which Ehrlich would not have been able to do since the range of autoimmune diseases was not yet defined, but we are still at the early stages of grappling with the solutions for autoimmunity, which is just about where Ehrlich was in his thinking over a century ago (1).

The reviewer approaches the subject of B cell tolerance to self antigens with some degree of trepidation because B cell development in its ability to recognize and respond to foreign antigens is still incompletely understood. Developing B cells at various stages of their development respond to self antigens with survival on one hand and removal or muting on the other hand. Therefore, the question is not simply how does one promote B cells recognizing foreign and eliminate or suppress B cells recognizing self. The question may be how the recognition of self promotes useful B cells and removes non-useful B cells regardless of whether foreign or self is recognized. This conundrum is somewhat like the one T cells experience when T cells have to bind self-MHC for MHC-restriction but not react and thus be eliminated as self-reactive cells. Do B cells go through a similar process, either binding to self antigens but not responding, or becoming connected to anti-idiotypic antibodies? To go back one more step, how much selection of B cells at various stages on the basis of their BCRs is ligand-dependent and how much is ligand-independent (8)? A recent study in a BCR-surrogate B cell model suggests further that development towards B-1 B cells requires stronger signals than adequate for development towards follicular and marginal B cells, and that these pathways may not necessarily require the action of T cells (9).

3. CURRENT STATUS

The ideas that B cells loose the ability to respond to self antigen at a particular stage, have a single tolerant phenotype and possess a single tolerance-inducing mechanism are less accepted now than they were twenty years ago. B cells become non-responsive to self antigens at different epochs of their life stages. Indeed, the singular ability of B cells to alter through somatic hypermutation (SHM) the antigen-binding sites of their BCR until late in

antibody responses to antigen requires that B cells must maintain many mechanisms for dampening anti-self responses. There are likely to be different mechanisms that can operate most efficiently at various stages of B cell development and responses to antigen. The view that removal of anti-self potential is the sole property of early signaling events, such as initial scaffolding (10), does not accord with our current understanding of B cell function and how B cells control immune responses to host antigens. Indeed, the generation of anti-self antibodies in autoimmune susceptible mice requires receptor editing (11), so that this process, which is usually considered to be a mechanism to avoid responding to self antigens, may be required to generate autoimmune B cells. The continued ability to develop autoimmune specificities has to be countered with control mechanisms that operate at various stages of B cell development and responses to antigens, and may become defective in autoimmune hosts.

It is difficult to enunciate clear universal rules regarding B cell non-reactivity to self. One generally accepted rule is that widely available self-antigens induce states of B cell non-responsiveness and the lack of antibodies to these self antigens. This is the case for the ABO antigens of erythrocytes or for certain serum proteins to which B cell responses and antibody production are muted. However, this rule is not obeyed in all situations in that efficient apoptosis of some antigens leads to a transient immune response, whereas the continued presence of self-antigen due to defects in apoptosis may lead to autoimmunity (12).

Without the expression of the antigen as self, immune responses can be induced either naturally or by intentional immunization. Perhaps some of these responses are of the treadmill variety where host antigens remove and metabolize antibody without being damaged (13, 14), a process seen with bacterial carbohydrates (15). Here, the hyporesponsiveness is more apparent than real. Other readily available self antigens can stimulate B cells and result in the production of antibody that is easily detectible. A notable example is the production of rheumatoid factor (RF), an IgM antibody directed to the Fc portion of IgG. Two suggestions are that the fine specificity of RF is really alloimmune rather than autoimmune, or that the form of the autoantigen is as aggregated IgG found in tissues rather that of monomeric IgG found in circulation. The B cell response to autologous IgG resulting in the formation of RF may involve the simultaneous activation via BCR which recognizes IgG in a complex and TLR9 that binds hypomethylated CpG motifs in the complexes (16). Whether or not normal B cells (non-transgenic and non-autoimmune) can be activated by such stimulation remains to be demonstrated.

Classical experiments, using non-transgenic mice that either produced or did not produce the fifth component of complement (17, 18) as a self antigen, showed that B cells were not made tolerant in the presence of the self-antigen. The study from Borel's group (17) concluded that (a) natural tolerance to C5 is an active process that is T cell dependent and requires the presence of antigen; (b) in this natural model, clonal abortion does not seem to occur; and (c) both tolerant and non-tolerant B cells retain the capacity to produce autoantibody. Stockinger's study (18) demonstrated that non-tolerant T cells would allow the induction of antibody in B cells that developed in the presence of the self-antigen. Thus both of these classic studies favored the concept that B cells were not deleted or made anergic in the presence of a self-antigen, but only required non-tolerant T cells to produce antibody.

One should contrast the conclusions reached in these classical experiments with those of a triple transgenic model in which mice are transgenic for anti-hen egg lysozyme (anti-HEL) BCR, for anti-HEL-peptide TCR, and for HEL antigen (presumably for both intact HEL and HEL peptides made available by proteolytic activity) (19). B cells from the BCR/TCR transgenic mice chronically exposed to HEL during their development did not produce antibody, but were eliminated through a Fas-dependent mechanism in the presence of HEL-specific CD4+ T cells. The difference in outcomes between this experimental system and that described in the previous paragraph could not be more marked. In the classical experiment (17, 18), T cells could rescue responses in autoantigen-exposed B cells, whereas in the transgenic experiment (19), T cells delivered the coup de grace to autoantigen-exposed B cells. It does not appear that this contrast in outcome between these two experimental approaches has been discussed or even acknowledged.

3.1. Venues for studying B cell tolerance to self antigens

Because of the difficulty with studying normal lymphoid tissue to determine the nature of B cell quiescence to self antigens, the most common current way to study B cell tolerance to our own antigens is to study the development and behavior of antigen-specific BCR-transgenic B cells. The distribution of antigen can be controlled so that a foreign antigen can function as a neo-self antigen, being present in the host either ubiquitously or as a tissue specific antigen. With such systems it is easy to observe the deletion of anti-self BCR-transgenic B cells, and the interpretation is that this deletion is what happens in non-transgenic conditions with self-reactive B cells. The problem with this experimental model is that one can demonstrate BCR-transgenic cells at early stages of B cell development (20) when normal cells would not have yet produced a self-reactive BCR. It is not clear to this reviewer if any BCR transgenic model, used for assessing the disposition of anti-self B cells, completely lack the expression of transgenic BCR up until the point in B cell differentiation when BCR would be normally expressed.

Thus current transgenic models may really explore what may happen during a point in B cell differentiation when the preBCR is normally downregulated by its aggregation, thus allowing B cell activation to be followed by a return to a resting state (21). Transgenic anti-self BCR aggregated by self antigen may not be downregulated at this stage of B cell development and disturb B cell development. We do not know what happens to B cells that cannot downregulate their preBCR, but it is a reasonable guess that the well-ordered activation

and return to a resting state may be replaced by B cell dysfunction and elimination. To date, no model of forced expression of the preBCR has been generated to study the effect of continued expression of the preBCR. An experimental model in which Blk, the src-related kinase activated by the preBCR, is constitutively activated to allow preB cells to advance to the next developmental stage (22) does not answer this question, because Blk may show prolonged activation normally even though the expression of surrogate light chains and the preBCR is rapidly down-regulated.

The reason for developing transgenic and superantigen models for studying lymphocyte unresponsiveness to self is that current methodologies do not allow biochemical analysis of this process in naturally developing B cells, because the anti-self B cells are heterogeneous and are present in small numbers among a vast majority of other B lymphocytes. In an ideal world, what is needed are methodologies to discern normal anti-self B cells while they are in the process of becoming hyporesponsive to self and later when they have become hyporesponsive and non-damaging. There are, therefore, two experimental requirements. First is to be able to study the cellular biochemistry of B cells that represent a small minority of the total B cell population. Second is to distinguish between the biochemical events associated with the induction of tolerance to self from the biochemical characteristics that mark a B cell that has become tolerant to self, when it again encounters self.

Another approach has been to clone cells from various stages of the B cell lineage and test them for reactivity against potentially self antigens, mainly intracellular antigens (23). Using this experimental procedure, the transition, firstly from early immature B cells to immature newly exported B cells from bone marrow to the periphery, and secondly from immature to mature B cells in the periphery are two potential checkpoints for the reduction in anti-self reactivity at least as a percentage of the total population. Of these two checkpoints, the first one entails the greatest reduction in the number of cells and part of this reduction may be the removal of autoimmune B cells. But other reasons for loss of B cell intermediates may be malformed or badly associating heavy and light chains and other possible developmental defects. In some studies, the quantity of cells that disappear at the various transitional B cell stages from immature to mature B cells was found to be relatively minor and therefore did not favor a significant culling of autoreactive B cells (24); furthermore the mature B cells maintained their multireactivity and autoreactivity. However in other studies (25), the attrition during the immature to mature B cell transition in the periphery appeared substantial, so that culling of self-reactive B cell may have occurred.

Mature B cells display a percentage of anti-self reactivity similar to that of early immature B cells provided that they naturally express surrogate light chains (26). This would appear to answer the question posed earlier in this section, but does not, since the preBCR may have been downregulated normally and then the surrogate light chain re-expressed later at the mature B cell stage. Assuming the latter, the surrogate light chain may be expressed preferentially in B cells with anti-self reactivity; however, the reason for this would be obscure in that it is difficult to see how surrogate light chain expression would decrease the amount of receptor crosslinking in these anti-self B cells. The other possibility is that surrogate light chain re-expression is a random process that allows a proportional selection of the general population of mature B cells that can be detected in the experimental system used; this general population of mature B cells would have maintained its normal degree of anti-self reactivity.

3.2. Signal 1 and the many forms of Signal 2

The polar events of B cell responsiveness and B cell tolerance was initially thought to be determined by whether or not two signals occurred. Signal 1, through the antigen-receptor, induced tolerance and this could be overcome by Signal 2 from another cell. For B cells this other cell was the helper T cell and the resultant cell-cell collaboration. The molecular mechanism for most immunologists has become the interaction of CD40/CD154, a receptor-ligand or receptor-receptor pair that activates additional signaling pathways. While initially exclusive, the second signal has been broadened to include other mechanisms. Furthermore, distinctly new activating signals have been added. Some of these costimulatory signals are based on cell-cell interactions and other costimulatory signals may be soluble factors, obtained from collaborating host cells and from microbial sources. While not yet clearly positioned in the B cell stimulatory scene, other activating signals such as those via toll-like receptors (TLR) (27) and via the tumor necrosis factor receptor (TNFR) family (such as BAFF-R, TACI, BCMA, etc) (28) play distinct roles in B cell development and responses. The interaction between BAFF and BAFF-R favors the survival of transitional and mature B cells, and the expression of BAFF-R is induced by BCR signaling events (25). These additional signals also seem to be of importance in the generation of responses to self antigens (29). The general idea with Signal 1 and Signal 2 is that Signal 1 to cause deletion or inactivation is a simple signaling event, while Signals 1 and 2 to induce a response is a more complicated event.

3.3. Inhibition by Signal 1 is reassigned to coinhibition

A change over the past couple of decades has been the identification of inhibitory signaling devices first in B cells and then later in T cells and NK cells. These findings have opened to question the classical idea that an isolated inhibitory signal (Signal 1) via the BCR is responsible for the attenuation of B cell/antibody responses to self antigen (30). There are a large number of coinhibitory devices, many of which are expressed on B cells and some of which are equipped with immunoreceptor tyrosine-based inhibitory motifs (ITIMs) (31, 32). ITIMs become phosphorylated by protein tyrosine kinases, such as Lyn that is bound by the immunoreceptor tyrosine-based activation motifs (ITAMs) of the BCR and activated, and bind phosphatases that counteract phosphorylation events involved in BCR signaling. Because of this two-way

interaction, we have referred to the process as coinhibition rather than inhibition. However, some of the control of B cell responses may involve simple inhibitory mechanisms, rather than coinhibitory mechanisms, and the two processes should be distinguished with further study and not assumed to be one or the other. Absence of the FcgammaRIIB coinhibitory receptor predisposes to autoimmune disease (33) and the steps in B cells that are regulated appear to be multiple (34).

The tendency has been for individual immunologists to focus on one of these inhibitory/coinhibitory signaling processes to the exclusion of the rest. Again, this does not accord with the functional requirements of regulatory control throughout the many stages of B cell responses. A B cell with no specificity for self antigen may begin by responding to a foreign antigen and then modify its BCR specificity through SHM to become reactive to self antigen. The control of these autoreactive cells well after their stimulation by foreign antigen may require mechanisms appropriate to this stage of response moving towards the formation of effector antibody-producing cells. Effective inhibitory/coinhibitory signaling in initial B cell responses to self antigens does not preclude the requirement for effective inhibitory/coinhibitory signaling at later stages of B cell responses to self antigens. Even when considering one stage in B cell differentiation, one inhibitory/coinhibitory signaling mechanism does not preclude another. An example is the distinct regulatory functions of Fas (CD95) and FcgammaRIIB (CD32); when both are defective the result is an aggressive B cell autoimmunity in one experimental construct (35). Fas is an apoptosis-inducing receptor whereas FcgammaRIIB is a coinhibitory receptor that may or may not promote apoptosis. This interaction may also be thought of as a kind of epistasis in which the defect of one component can be expressed in the presence of a defect in the other. Furthermore, the autoimmunity due to defects in both Fas and FcgammaRIIB may be dependent on other parts of the genome depending on the background strain of the experimental model or on the fine specificity of the experimental construct; epistastic events may occur at multiple levels.

The idea that different coinhibitory elements may regulate different processes in B cell function does not preclude the possibility that a single coinhibitory element may regulate different stimulatory processes. An example is that FcgammaRIIB activation seems to disturb both B cell activation (36) and antigen-processing for T cell help (37, 38). Nor does the definition of an ITIM-based mechanism for inhibition by a given receptor preclude the operation of other mechanisms (39). Simplistic pronouncements on complex situations, the ‘axis of evil’ approach, lead immunologists into their own little Iraqs from which they have difficulty escaping.

ITIM-bearing coinhibitory receptors currently found on B cells include FcgammaRIIB, CD22, CD5 (on B-1a), CD72, PIRB, ILT, IRTA1, PP14, and others. Without reviewing each of these receptors, suffice it to state that B cells are well endowed with coinhibitory receptors that could regulate the activity of anti-self B cells. This leaves open the question of how these receptors control B cell responses to self antigens, but do not interfere with protective immune responses to the antigens of infectious agents. The bottom line with regard to the negative control of anti-self B cells is that the process is a complex process that influences and is influenced by costimulation (31); it is not primarily the result of Signal 1, a simple inhibitory signaling event via the BCR.

3.4. Receptor editing serves as a mechanism for B cell tolerance to self antigens

The major mechanisms for avoiding damaging anti-self immune responses were thought to be deletion, anergy, ignorance and regulation. Both heavy and light chain genes are capable of utilizing alternate genes through a process of receptor editing. The light chain V and J genes have the option for further recombinations because of additional 5-prime V genes and 3-prime J genes (40). The joining of V, D and J genes does not freeze the complete heavy chain variable region gene because a heptamer is embedded within the complete VDJ to allow the incorporation of other V genes. For such recombinations, the recombination enzyme mechanisms must be expressed. This normally occurs during heavy chain VDJ and light chain VJ gene recombination, but the recombination enzymes are expressed during B cell activation by antigen and at the time of SHM (41, 42). Therefore the VJ exons of light chain variable regions that develop the potential to react to self antigens can be edited out by de novo VJ recombinations. One assumes that the new VJ exon codes for a random specificity. This is intriguing in that the B cell in which this editing has occurred would be a primed B cell that has not yet seen the antigens to which it now may bind and respond. T cells have been shown to reactivate the V(D)J recombinase mechanisms, so that T cells with their inability to undergo SHM (or do they?) have recourse to the editing mechanism later in their life stages (43). Why should this be necessary, given that T cells do not undergo SHM or class switch recombination (CSR)? When naïve T cells are activated, they do change their complement of adhesion molecules; this change in adhesion molecules may allow TCRs with limited affinity for self antigens (TCRs are selected in the thymus on this basis) to be stimulated to generate damaging effector cells, and mechanisms to deal with this eventuality may be needed.

3.5. Intolerance in tolerance mechanisms

Historically, the major mechanisms for avoiding damaging anti-self immune responses were listed as deletion, anergy, ignorance and regulation. When individual scientists made their list, it often became clear that each scientist favored one of these mechanisms above the others. The desire was to have one mechanism for avoidance of immune responses to self that operated at a specific point of time (checkpoint) in the development or function of B cells. Scientists argued about mechanisms and checkpoints, and strove to develop experimental models that supported their point of view against all other views. Even though it is conceptually impossible that B cells can remove anti-self immune responses until the formation of a complete BCR, some favored deletion even

at a time prior to the point of the generation of a complete BCR with a defined specificity. This was most evident in transgenic or superantigen models in which the expression of a transgenic antigen-receptor was forced at a stage at which the natural antigen-receptor is not normally expressed, or because the selecting antigen did not use the antigen-receptors specificity, as in superantigen models.

Another prejudice was that non-deletion mechanisms were inherently unstable because anergic, ignorant or regulated B cells could be reactivated to cause immune damage. However, one can argue the reverse. The deletion of anti-self B cells could leave the immune system with a 'hole' that could be filled later by autoimmune B cells; no effective regulatory mechanism would be available to deal with these cells. Anergic, ignorant or regulated B cells may themselves be capable of regulation so that they can deal with newly arising autoimmune B cells. This reasoning suggests that non-deletion mechanisms may be inherently more stable. Furthermore, B cells that have been regulated may only allow antibody production to a low and non-damaging level, the establishment of a set point.

3.6. B cell tolerance mechanisms are a 'both/and', not an 'either/or'

The numbers of deficiencies in B cells leading to an autoimmune problem are increasing in number. This suggests that the avoidance of damaging responses to self antigens must be assured again and again for B cells. Part of the reason for this may be that modification of the BCR continues into late stages of B cells, after they have been stimulated by antigen and have received costimulatory signals from T cells. It would be of interest to compare the locations of tolerance-inducing mechanisms for T cells and B cells; my prediction is that the tolerance inducing mechanisms for B cells may occupy a broader range of B cell life stages than that of T cells. Without SHM, tolerance-inducing mechanisms would not be as crucial for late stages in the life of T cells, as they are for B cells. T cells would have settled the anti-self reactivity earlier in differentiation and not be forced to remove anti-self specificities arising later through SHM. However, the question of variation of cell adhesion molecules on naïve versus memory T cells, mentioned earlier, as well as T cell subsets may vex this simple prediction. Another question is do tolerance-inducing mechanisms occur in early B cell life stages if the late life stages must have them also? It will be interesting to see if bone marrow possesses a mechanism of presentation of tissue specific antigens analogous to that of autoimmune regulator (AIRE)-mediated mechanisms evident in the thymus. While there are a number of transcription factor defects associated with B cell autoimmunity (44-46), none involves the expression of peripheral tissue-specific antigens in bone marrow, the site of B cell development.

4. FURTHER CONSIDERATIONS ON THE B CELL UNRESPONSIVENESS TO SELF ANTIGENS

While little direct information about the status of natural B cells to host antigens is available, it is worth some more discussion regarding the characteristics of the unresponsiveness.

4.1. How much does affinity count in determining B cell tolerance to self antigens?

In terms of anti-self immune responses, it was suggested that low avidity anti-self B cells would be accepted into the B cell pool, from which high avidity anti-self B cells would be eliminated. This does not appear to be the case, at least in transgenic models. Within the structure of B cell-membrane rafts, even low affinity multiple BCRs may generate a high avidity for multivalent antigen. In one transgenic study, a 10,000 difference in affinity of the BCR for antigen had a surprisingly little effect on ability of multivalent antigen to censor the B cell (47). However, in a more recent study, dilution of anti-self transgenic BCRs with normal BCR, or transgenic BCRs that do not react to self, protected from deletion by membrane-bound transgenic antigen (soluble antigen did not cause deletion) and, indeed, allow for positive selection of these double BCR B cells against the self antigen (5).

4.2. Are all anti-self B cell responses and antibodies nasty?

The answer appears to be no, since the removal of effete cells may involve the expression of components to which the host's immune system is not tolerant. Attached antibody would increase the rate of phagocytosis and removal of effete cells. There is also the possibility that some anti-self B cells recognize antigens peculiar to cancer cells. Autoimmunity to proinflammatory cytokines helps in preventing damage to self (48), thus autoimmunity (autoantibodies to proinflammatory cytokines) may protect against destruction of self tissues by anti-self immune responses.

4.3. B cells as APC and regulatory cells

Naïve B cells are the precursors of antibody-forming plasma cells and memory B cells. They also have the capacity to present antigen and are, therefore, antigen-presenting cells (APC). B cells also have clearly defined regulatory functions and produce a number of cytokines and chemokines. How much this plays into the control of immune responses to self is not clear. While abnormally present autoimmune T cells may activate naturally occurring autoimmune B cells, the reverse may also happen. In a number of autoimmune diseases in which the effector mechanism is clearly T cells, removal of B cells may prevent the T cell-based autoimmunity, suggesting that B cells have antigen-presenting and regulatory functions.

4.4. Do B cell functions have a beginning and an end?

B cells are generally considered to develop, respond to antigen, form effector cells plus end products and memory cells, and then much of this population disappears when the antigenic stimulus subsides. In this linear time scale, there does not seem to be much of an opportunity for antibody to influence early steps in B cell responses to antigen, either foreign or self. This is particularly so for IgG antibody, which requires class switch recombination to be produced in large amounts. However, many hosts display antibody before B cells begin

to function, having obtained it from their mothers. Furthermore, it is instructive to look at the specificities of monoclonal antibodies. While generally specific, these antibodies can have unexpected crossreactivities with seemingly unrelated antigens. The basis for this crossreactivity appears to be that unrelated antigens may have small patches of similar structures recognized by the antigen-binding sites of monoclonal antibodies. The exquisite specificity that we may see in antibodies is population-based, due to the summation of specificities of many antibodies to a given antigen. Therefore, it would be unwise to conclude that hosts naïve to a given antigen would be totally devoid of antibodies to it, including IgG antibodies. Antibodies to both foreign and self antigens may exist at low but regulating amounts prior to encounter with a given antigen. Assigning regulatory mechanisms to distinct steps in B cell development and response will not be an easy task. This message has been driven home again by the finding that class switch recombination may occur during B cell development in the bone marrow (49).

5. WHAT CAN WE LEARN FROM B CELL AUTOIMMUNITY?

5.1. Differences in anti-self reactivity between B-1 and B-2 cells

There are two categories of B cells, B-1 and B-2. So far we have been discussing the B-2 cells, the majority of B cells that display the classical features and capacities of B cells that form the adaptive humoral immune response. B-1 cells utilize germline encoded sequences without modification and produce IgM antibodies that are polyreactive and autoreactive (50). B-1 cells are stimulated poorly by ligation of their BCR (51, 52). The antibodies produced by B-1 to various nominal antigens do not require prior exposure to external antigens (53), so that these antibodies are considered 'natural'. Normally the B-1 population is exposed to self antigen and is only capable of low, non-damaging, levels of autoantibody production because of negative signaling devices, such as CD5. When self antigen does not contact B-1 cells, they may produce damaging levels of antibody but this represents an experimental phenomenon. Natural antibody may aid in the induction of immune responses because it can bind antigen, aggregate antigen and activate complement; thus it favors both phagocytosis and antigen-presentation and activation of B-2 cells by causing greater aggregation of BCR and co-aggregation of BCRs with the CD21/CD19/CD81 co-receptor complex. The big mystery here is why the autoreactive aspect of B-1 antibody does not make it more likely to induce autoimmunity. It seems to do the opposite, if B-1 derived autoantibodies are responsible for the clinical benefit of intravenous immunoglobulin (IVIg) in the treatment of a number of autoimmune diseases (54). The inhibitory action of IVIg requires the inhibitory FcgammaRII (55), although the mechanism of this inhibition is currently debated (56, 57).

5.2. Do autoimmune B cells cross a Rubicon?

Initially, autoimmune damage was thought to be caused by the production of 'forbidden clones'. If these anti-self lymphocytes where produced by some abnormal process, due to a lack of deletion, damage to self would be evident and persistent. However, with the finding that a certain level of anti-self B cells was not associated with damage to self lead to the concepts of anergy and immunologic ignorance. While most attacks against self antigens are persistent, leading to chronic autoimmune diseases, some are not. A good example is Goodpasture's disease in which an antibody responses against a collagen component in basement membrane causes renal and lung disease. This disease can be rapidly damaging and fatal, but, if it is adequately treated, the disease process is broken and recovery without further use of immunosuppressive agents is the rule. Some diseases caused by immune responses to self antigens are monophasic; the outcome is not only quiescence but resistance to further attempts to induce of autoimmune disease.

5.3. Pure T cell autoimmunity results in production of many autoantibodies

Autoimmune polyendcrinopathy-candidias-ectodermal dystrophy (APECED) is caused by defects in the autoimmune regulator (Aire) a transcription factor that promotes the ectopic expression of tissue-specific antigens in the thymus that normally results in central deletion of autoreactive T cells (58, 59). In APECED, a wide range of autoantibodies are generated, as they are in immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX) disease in which the lack of FOXP3 leads to a defect in production of regulatory T cells (60). This suggests one of two possibilities. Autoreactive B cells are not normally made tolerant to peripheral autoantigens and only require the development of autoreactive T cells or some other costimulatory process (61-63) in order to become activated. The other possibility is that the presence of autoreactive T cells interferes with the normal attainment of tolerance to self antigens within the B cell population (64). One could conclude that these B cell responses are due to a generally disturbed immune system, however, an analogous phenomenon is seen in celiac disease. Here, antibodies to host transaminase are generated because ingested gluten and host transaminase forms a stable hapten-carrier complex that transaminase-specific B cells endocytose; these B cells then present gluten peptides to gluten-specific T cells that help the transaminase-specific B cells respond to host transaminase. That this sort of event occurs does not indicate that anti-self B cells are never eliminated or made non-functional, but that anti-self B cells to some self antigen remain and are capable of activation when the conditions are right.

The loss of B cell non-reactivity to self in response to T cell activity appears to involve at least two steps (65). The first step is activation of B cells to form autoantibody of the IgM type and movement of the B cell into the germinal center. The second step is further activation of T cells so that they can move into the germinal center and collaborate with B cells there to induce SHM, affinity maturation and class switching to IgG production. While interfering with the entry of T cells into germinal centers may be a possible approach to autoimmunity, autoimmune-prone mice seem to be able to carry out some of these processes outside of the germinal

center (66). Interference with T cell activation by costimulatory signal blockade seems to be useful in preventing experimental autoimmunity in disease-prone strains of mice (67).

6. PERSPECTIVE

The normal lack of damaging B cell responses to self antigens is the product of many mechanisms that interact in complex and often unpredictable ways. These interactions cannot be reduced to a simplistic mechanism, such as those expressed in minimal models of self-nonself discrimination, such as the various two signal models. Experimental models that study mechanisms for the control of anti-self B cell responses elucidate a facet of multifaceted regulatory networks, but do not reduce such networks into a one-dimensional process. The function of regulatory mechanisms that impact strongly on the control of the immune system should be positioned conceptually so that they interact with other regulatory systems previously shown to be of importance in the control of immune responses to self antigens. While current methods have given us partial insights into the control of B cell responses to self antigens, a full understanding will await the development of methods that allow the study of processes under natural, non-manipulated, conditions in which the induction and maintenance of tolerance to self antigens can be observed directly.

The rise in damaging immune responses to self antigens in western societies has told us something profound about the immune system. While mechanisms such as molecular mimicry, bystander activation, and supplemental immune mediated tissue destruction may occur in infections, these are seemly outweighed by events in infection that decrease rather than increase the risk of damaging reactions to self antigens. The immune system regulates itself better when used in the defense of the host. Part of the reason may be the generation of a larger population of regulatory cells. However, concepts such as homeostasis and competition for limited resources (5) may also shed light on why there is an inverse correlation between infections and the occurrence of autoimmune diseases. Immune responses to self antigens tend to occur in areas where sustaining resources are sparse, while immune responses to infecting agents occur in areas endowed with more resources to initiate, amplify and sustain immune responses.

We have moved some distance from the concept of forbidden clones and that the generation of anti-self lymphocytes leads inexorably to autoimmunity and to autoimmune disease. Not only does competition operate on cells newly entering the peripheral cell pool, cells that have been experienced a suboptimal response or become tolerized by self antigens have reduced ability to compete for resources, including the ability to enter and function within germinal centers (68). At one time, the logic was that the earlier in lymphocyte development a control mechanism was, the more powerful it was in regulating the immune response. More recently, mechanisms that operate late in immune responses to antigens have been identified that have important roles in the control of immune responses to self antigens. Some of these include limiting the responses to self antigens, but others involve limiting responses to foreign antigens once the threat from these microorganisms expressing these antigens has disappeared. When the mechanisms for this limitation are not working (such as with defects in apoptosis) or are overbalanced by the forced expression of competing processes (such as anti-apoptotic proteins) then autoimmunity may occur.

Some decisions about survival and responses are made in individual B cells, but many other decisions come from the interaction of the population of B cells with other cells and themselves (69). Decisions may be the wrong word, and tendencies may be a better one. B cells move into a state where a certain set of outcomes becomes more likely. The longer B cells remain in a particular state or range of states, and the more this condition is reinforced, the more likely a certain range of outcomes will occur. For proper development and function, B cells sample from a series of trophic environments. B cells, which get stuck in one environment because they have a BCR specific for and inappropriate for host antigens in that one environment, will not properly sample from the range of trophic environments needed to maintain proper development and function. These B cells will tend not to compete well with B cells that have received the appropriate trophic signals at the appropriate time and stage in their development and function. The receipt of appropriate trophic signals would depend on these signals and their receptors, on various adhesion and chemokine ligands and receptors, but also on the affinity of the BCR for self antigens that would encourage the proper placement, signaling and survival of B cells. Like the tale of the Three Bears, too little or too much would be detrimental, while just right would allow proper development of B cells that have the best chance to respond appropriately to environmental antigens.

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