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Establishing Anergy as a Bona Fide In Vivo Mechanism of B Cell Tolerance

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The pioneering work of Owen (1) and Burnet and Fenner (2) in the late 1940s originated the concept of immune tolerance. Subsequent experiments conducted by Billingham et al. (3) first demonstrated the active induction of immune tolerance using a skin graft rejection model. These authors consequently coined the phrase “actively acquired immunological tolerance.” The findings of Billingham et al. were put into structural perspective by David Talmage’s hypothesis of cell selection (4) and Burnet’s clonal selection theory (5), both published in 1957, which provided a framework for understanding how it might be possible to maintain tolerance to self while remaining responsive to foreign Ags. Specifically, if one B cell encoded a single specificity, deletion of self-reactive cells would provide tolerance while sparing responses to other Ags. An important qualification of the clonal selection theory was proposed by Joshua Lederberg (6), who postulated that lymphocytes traverse a developmental stage during which they are uniquely sensitive to tolerance induction. Of course we now recognize that this principle applies to so-called “central tolerance,” wherein autoreactive cells are silenced by deletion (T and B) and receptor editing (B) that occur in the bone marrow and thymus. Gradually, additional silencing mechanisms were defined, such as those involving suppressor/regulatory T cells, idiotypic regulatory networks, and anergy. Silencing of autoreactive B cells by anergy was first established unequivocally by the Pillars of Immunology article (7) that is the subject of this commentary.

Among the earliest indications that B cells per se could be rendered tolerant were the experiments of Siskind and colleagues in 1963 (8) in which pneumococcal polysaccharides were used as tolerogens/AgS. The “thymus independence” of responses to this Ag made it unlikely that T cells were involved in silencing the response. Subsequent in vitro studies using isolated B cells clearly demonstrated that these cells could be rendered tolerant (9–12). Consistent with the hypothesis of Lederberg, these studies also showed that immature B cells were more sensitive to tolerance induction than mature B cells, requiring 1000-fold less Ag than mature splenic B cells to achieve tolerance (9).

In early studies, the mechanism by which B cells were tolerized was often referred to as clonal inactivation/abortion. It was shown that the frequency of Ag-specific, Ab-producing cells fell drastically following tolerization treatments. However, these experiments did not discriminate whether this was due to clonal deletion or to the induction of a state in which the cell persisted but was simply unresponsive to Ag. To address this question, Pike and Nossal used a fluorescein (Flu)–human gamma globulin (HGG) tolerization protocol in conjunction with analysis of both the capacity to produce Ab responses and enumeration of the Flu-specific B cells (13). They found that in mice treated neonatally or in utero with high doses of Flu-HGG, there was a significant reduction in the number of Flu-binding cells, suggesting clonal abortion/deletion. However, when they used low Ag doses they found no reduction in the number of Flu-binding cells or their avidity profiles; nevertheless, the cells did not produce Abs upon stimulation. They coined the term clonal “anergy” to describe this B cell unresponsiveness.

Although the conclusions of Pike and Nossal would be proven correct, there were some caveats in their experimental approach. For example, their reported Ab-forming cell precursor frequency was much lower than would have been predicted based on the Ag-binding cell frequency. Therefore the differentiated daughters of Ag-binding cells remaining after tolerance induction may never have been capable of producing measurable anti-Flu responses, e.g., prior to tolerance induction. Moreover, like all previous B cell tolerance studies, the approach involved tolerization with exogenous foreign Ag in contrast to the physiological situation in which the self-Ag would be present throughout the ontogeny of autoreactive B cells. Finally, the tolerogen used in the studies, Flu-HGG, would bind the inhibitory IgG constant receptor FcyRIIB expressed by the B cells, and this could suppress the subsequent immune response. Thus, although Pike and Nossal originated the concept of anergy, Chris Goodnow and his mentor Anthony Basten (7) proved that anergy is operative in the silencing of autoreactive B cells in vivo.

As reported in the featured publication, Goodnow and Basten et al. (7) took advantage of the then newly developed transgenesis technology to express hen egg lysozyme (HEL) as a neo-autoantigen in mice. The Ag was expressed under a metallothionein promoter to facilitate expression from embryonic to adult life. Furthermore, use of this promoter allowed the modulation of Ag expression by exposure to heavy metals. In these mice (ML5 line), the expression of HEL induced tolerance in both HEL-specific B and T cell populations, confirming that it was recognized as self. ML5 mice...
were then crossed with a B cell Ag receptor transgenic line (MD3) that expressed a BCR with a high affinity for HEL. The IgM and IgD of the MD3 mice were of the IgH\(^\alpha\) allotype that, when on an IgH\(^\beta\) background, allowed tracking of the MD3 B cells and production of IgM\(^\alpha\) by these cells or their daughters.

The results of the experiment were striking. The presence of HEL did not lead to the deletion of most MD3 B cells; in fact, they were present in the periphery in nearly normal numbers but exhibited reduced surface IgM levels. Interestingly, IgD expression remained high. Although MD3 mice had high spontaneous IgM\(^\alpha\) anti-HEL titers, MD3 \(\times\) ML5 double transgenic mice produced barely detectable levels of IgM\(^\alpha\) anti-HEL Ab. Moreover, in an adoptive transfer experiment in which either MD3 or MD3 \(\times\) ML5 spleen cells were transferred into sublethally irradiated C57BL/6 mice together with HRBC-primed T cells, HRBC-HEL immunization led to drastically lower anti-HEL responses in recipients of MD3 \(\times\) ML5 spleen cells than in recipients of MD3 spleen cells.

These results grounded the clonal anergy theory. A year later, David Nemanzoe and his colleagues would demonstrate clonal deletion of autoreactive B cells in a BCR transgenic model specific for MHC class I (14). This study would be followed by another paper by Hartley, Bosten, and Goodnow showing that B cells that became anergic in the presence of soluble HEL were deleted when the MD3 mouse was crossed to a mouse expressing HEL in a membrane-bound form (15). This result underscored the importance in B cell fate determination of the form of self-Ag that is encountered (i.e., monomeric/oligomeric soluble vs highly multivalent membrane bound). As stated by Hartley et al., “...signaling through the B-cell antigen receptor is not an all-or-none event, but...quantitative differences in the degree of receptor crosslinking may bring about qualitatively different cellular responses.”

Subsequent studies using the HEL-anti-HEL and other transgenic models provided a number of additional important insights regarding B cell anergy. By using transgenic models, it was possible to determine the relationship between the degree of receptor occupancy (a function of receptor affinity, autoantigen concentration, and total avidity) and the fate of autoreactive B cells. In the anti-HEL model, occupancy of <5% of receptors led to development of B cells that were “ignorant” of Ag and >5–45% receptor occupancy to anergy, whereas high occupancy, particularly in conditions of high Ag avidity, led to deletion (16). In another transgenic anergy model in which B cells were specific for arsenate but displayed low-affinity crossreactivity for ssDNA, the so-called Ars/A1 model, occupancy of ~20% of receptors led to anergy (17).

Another key question related to potential changes in tolerance susceptibility during development was this: did the Lederberg hypothesis apply to anergy? Regardless of their maturation state, the transfer of nontolerant MD3 anti-HEL B cells into an environment with appropriate levels of HEL led to anergy (16).

Another important question that is now being debated, particularly in view of reported genetic reprogramming in anergic T cells, is whether the induction of B cell anergy involves some durable form of reprogramming or is reversible. The transfer of anergic B cells into an environment lacking HEL gradually (over days) results in loss of the anergic phenotype and reacquisition of the ability to produce Ab responses to HEL (18). Complementary studies using the lower affinity Ars/A1 model indicated that the anergic phenotype is lost within minutes of the dissociation of autoantigen (19). Although the delay in the loss of anergy upon the transfer of MD3 \(\times\) ML5 B cells could be interpreted as resulting from a requirement for the decay of a transcriptionally regulated repressor, other data suggests this might be due to the relatively high affinity of the MD3 antibody for HEL (\(K_c = 2 \times 10^5\) M\(^{-1}\)) and thus slow dissociation of HEL from Ag receptors. Taken together, these data are most consistent with a requirement for the constant transduction of a BCR signal to maintain anergy (19).

The MD3 \(\times\) ML5 system established anergy as a mechanism of B cell tolerance. However, the model is unphysiologic in its reliance on transgenes and an Ag receptor possessing extremely high autoantigen affinity. Thus the studies did not establish absolutely the contribution of this mechanism to physiologic B cell tolerance in the wild-type repertoire. Goodnow did compare the phenotype of the B cells in MD3 \(\times\) ML5 mice with those in wild-type C57BL/6 mice and concluded that a population of IgM\(^\alpha\)/IgD\(^\alpha\) B cells exists in a normal repertoire, suggesting that this population could contain anergic B cells (16). Later studies by our laboratory and that of Darise Farris using a more extensive anergic cell marker set demonstrated the existence of anergic “An1” cells in the normal B cell repertoire (20, 21). More recently, an anergic B cell population has been identified in humans (22). Considering the estimation that up to 75% of newly formed B cells are autoreactive (23), the size of the An1 population, and the reduced life span of anergic cells, it appears that as many as 50% of newly produced B cells may be destined to become anergic (20). Thus, anergy is probably a major mechanism of tolerance and, particularly given its reversible nature, its failure is a likely source of autoreactive cells that participate in the development of autoimmunity.

Disclosures
The authors have no financial conflict of interest.

References


